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545. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

II. THE EFFECT OF THE PROTEIN CONTENT OF THE CONCENTRATES

BY C. C. BALCH, D. A. BALCH, S. BARTLETT,
C. P. COX, S. J. ROWLAND AND JILL TURNER
National Institute for Research in Dairying, University of Reading

(With 4 figures)

An earlier paper(1) described an experiment in which the fat content of milk from Shorthorn cows was depressed when they received only 6 or 2 lb. hay daily, with concentrates to supply the main nutritional requirements. Reported occurrences of this condition in other countries were reviewed. Attention was drawn to the lack of conclusive evidence that these depressions are due to a low intake of roughage *per se* rather than to an increased intake of concentrates. The results suggested, however, that the decline could be brought about without very large increases in the intake of crude protein. Since this point is of practical importance the effect of different levels of protein in the concentrates was investigated in a further experiment.

METHOD

Fourteen Shorthorn cows and one Guernsey were grouped in blocks of five so that the animals in each block had calved at about the same time. A fourth block of Friesians brought the total number of cows on the experiment to twenty. Five treatments, A–E, were allocated at random to animals within blocks, there being one animal on each treatment in each block. The experiment lasted 13 weeks with the following feeding programme.

Period 1 (preliminary control), weeks 1 and 2. All the cows received daily 18 lb. hay and a concentrate mixture balanced for milk production (mixture A) at about 4 lb./10 lb. milk produced. The cows received the same diet for 1 week before the experiment commenced.

Period 2 (period of experimental treatment), weeks 3–9. During the first week the diets of treatment groups B–E were changed gradually. When the change had been completed the twenty cows were receiving three treatments. Group A (four cows) continued to be rationed as in the initial control period; all the other cows received only 4 lb. hay daily, concentrates being given at the rate of 4 lb./10 lb. milk produced, with an additional allowance of about 6 lb. to compensate for the reduced hay; groups B and C (eight cows) received a concentrate mixture high in protein (mixture B), while the concentrates fed to groups D and E (eight cows) were low in protein (mixture C).

In weeks 7–9, cows in groups C and D received daily an addition of 10 lb. of delignified straw pulp (see below) supplying about 1 lb. crude fibre. This extension of the main

experiment was made as it was desired to take the opportunity of observing whether an addition of such fibre would affect the depressed milk fat content.

Period 3 (final control), weeks 10 and 11. The cows were rationed as in the initial control period, the change from the experimental diets being completed in 1 day.

Period 4 (grazing), weeks 12 and 13. Observations on milk composition were continued for 2 weeks during which the cows were grazing.

Foods. The hay consisted mainly of lucerne with a small proportion of cocksfoot, and had been well made on tripods. The constituents of the various concentrate mixtures are given in Table 1. A commercial mineral mixture was added to concentrate mixture A at the rate of 2 lb./100 lb. The straw pulp was prepared commercially from macerated wheat straw by digestion, under pressure, with caustic soda, followed by washing and screening.

Table 1. *The concentrate mixtures*

	Parts by weight		
Food	Mixture A (control)	Mixture B (high protein)	Mixture C (low protein)
Barley meal	—	3	3
Decorticated groundnut cake	3	6	1
Flaked maize	10	6	12
Maize gluten feed	—	3	—
Weatings	7	3	3

Table 2. *Composition of hay, concentrate mixtures and straw pulp* (%)

Food	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Ash
Hay	82·9	12·0	1·1	33·8	28·4	7·6
Concentrate mixture A	85·2	16·5	3·1	57·5	4·6	3·5
Concentrate mixture B	86·4	22·3	2·3	53·8	4·6	3·4
Concentrate mixture C	84·4	11·6	2·7	64·4	3·2	2·5
Straw pulp	14·2	0·3	—	3·5	9·8	0·6

The foods were weighed and sampled, and determinations of dry-matter content were made daily. The dry samples were bulked and then ground, mixed and analysed at the end of each period. The mean composition of the foods is given in Table 2; the conventional determinations were made by standard methods.

Management. The cows were housed at night in the cowshed, on sawdust bedding, and during the day were exercised on bare ground. They were fed from individual mangers which prevented the animals from stealing from each other and reduced wastage of food to a minimum; the cows were machine milked twice daily.

Milk. During each week three composite evening/morning samples of the milk of each cow were taken on alternate days. The fat content of the samples was determined by the Gerber method (2). The total solids content of the milk was determined gravimetrically (3, method 2) on one of the composite samples each week, and the solids-not-fat content obtained by difference.

Statistical analysis. Mean values for the yield of milk, milk fat and milk solids-not-fat, and for the percentages of fat and solids-not-fat in the milk, during the two control periods (weeks 1 and 2; 10 and 11) were used as concomitant variables in analyses of covariance for mean values over weeks 4–6 with three treatments (A; B and C; D and E) and for weeks 7–9 with treatments A, B and E. The effect of the addition of straw pulp to the diets was estimated by calculating the mean change during the period (week 7 to week 9) for

treatments A, C and D. The '*t*' test for values for milk yield, milk fat yield, solids-not-fat percentage and solids-not-fat yield, and the Fisher-Behrens test (4) for observations of milk fat content, were used to test the significance of these effects.

RESULTS

Food intake. In the two control periods the amounts of food left uneaten were negligible. Following the change to diets low in hay, nine of the cows refused 5 lb. or more of concentrates on 1 or more days. Loss of appetite was most common in the few days immediately after the dietary change had been completed, but in only three cows did it persist for more than 4 days and make necessary a reduction in the amount offered.

Milk yield. Mean daily milk yields of cows in the five treatment groups are plotted in Fig. 1. Two cows in group D gave much reduced yields on several days in week 4, but the experimental treatments do not appear to have affected milk yields and there was no statistical difference between the groups during the experimental period. The change to grazing caused increased milk yields in all the groups.

Fat content of the milk. Once the change to the diets low in roughage was completed there was a fall in the fat content of the milk relative to values for the control treatment. In weeks 4–6 this was not significant in groups B and C, but was significant in groups D and E ($P \doteqdot 0{\cdot}05$). At this time the fall in adjusted values for the groups receiving concentrates low in protein was significantly greater ($P \doteqdot 0{\cdot}02$) than that in the groups receiving concentrates high in protein. From Fig. 2 it can be seen that there was, however, a distinct tendency for values for groups B and C to fall during the experimental period, and in weeks 7–9 the fall in adjusted values for groups B (high protein) and E (low protein) were 0·47 and 0·72 % respectively, only that for low protein approaching significance ($P \doteqdot 0{\cdot}08$). The addition of 10 lb. of finely macerated straw pulp to the diet of groups C and D appeared to cause no real recovery in the milk fat content. In weeks 8 and 9 the fall in milk fat content (as compared with the control periods) exceeded 1·0 % in six and was less than 0·7 % in only two of the Shorthorns, whereas in neither the Friesians nor the one Guernsey did it exceed 0·08 %; the mean fall in the eight cows receiving concentrates high in protein was 0·67 % and in the group receiving concentrates low in protein, 0·72 %. The depressed milk fat content rapidly returned to normal after the change back to diets containing 18 lb. hay, but in all groups further increases in fat percentage were apparent during the period of grazing.

In an attempt to find the reason for the great variability of the changes in milk fat content the values for individual cows were examined. It was found that the depression in milk fat resulting from any experimental diets decreased as the stage of lactation of the cow, at the time of the change, advanced. To illustrate this point, in Fig. 4 the differences between the milk fat content of the cows in treatment groups B–E in weeks 2 and 8 have been plotted against the number of days the cows had been in milk at the start of week 8. The cows were blocked according to the stage of lactation, and the block of Friesians was the most advanced in lactation, and it was probably for this reason rather than because of any difference between breeds that no drop occurred in any of the Friesians. The Guernsey received treatment B, and although she had calved relatively recently, there was no fall in fat content. Comparisons between the results for the experimental treatment groups will have been less influenced by a stage of lactation effect, as the cows were blocked according to calving dates and allocated to comparable groups.

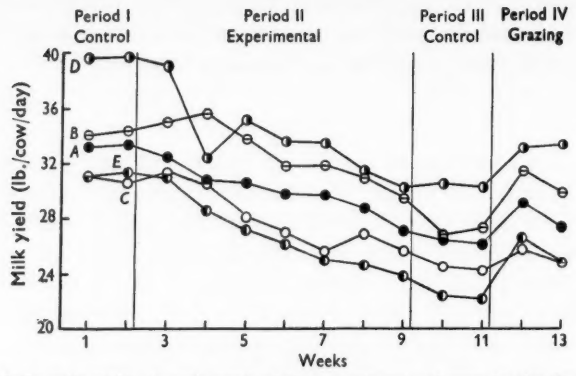

Fig. 1. The mean daily milk yield, in each week of the experiment, of cows in the five treatment groups. During the control periods all the cows received 18 lb. hay daily and balanced concentrates according to yield. During the experimental period cows in group A, ●, continued to receive this diet, but in all the other cows the daily hay intake was reduced to 4 lb., the other nutritional requirements of groups B, ⊖, and C, ○, being satisfied by concentrates high in protein, and of groups D, ◑, and E, ◐, by concentrates low in protein. In weeks 7–9 groups C and D received a daily addition of 10 lb. straw fibre.

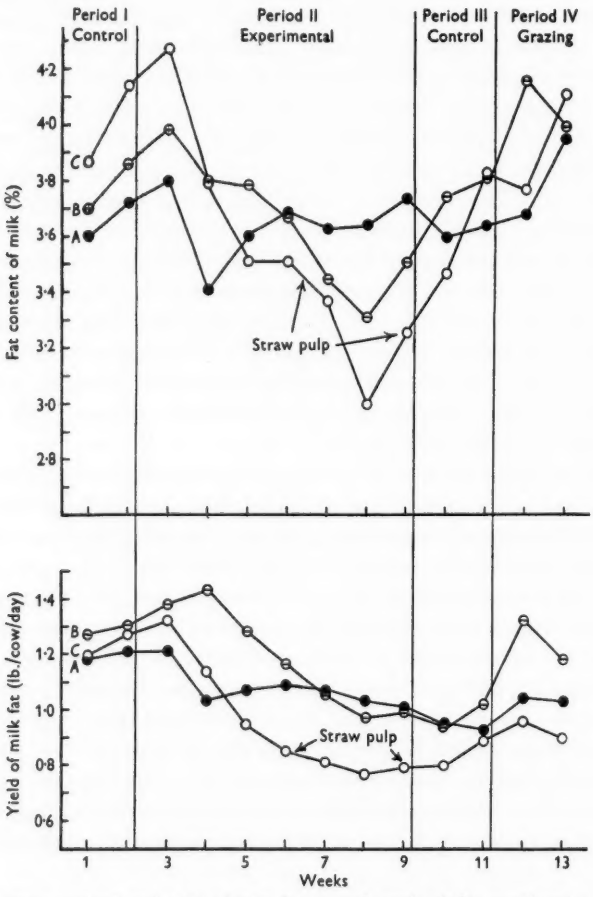

Fig. 2. The mean content and yield of milk fat, in each week of the experiment, of cows in groups A, ●; B, ⊖; and C, ○. The diets are explained in the legend to Fig. 1.

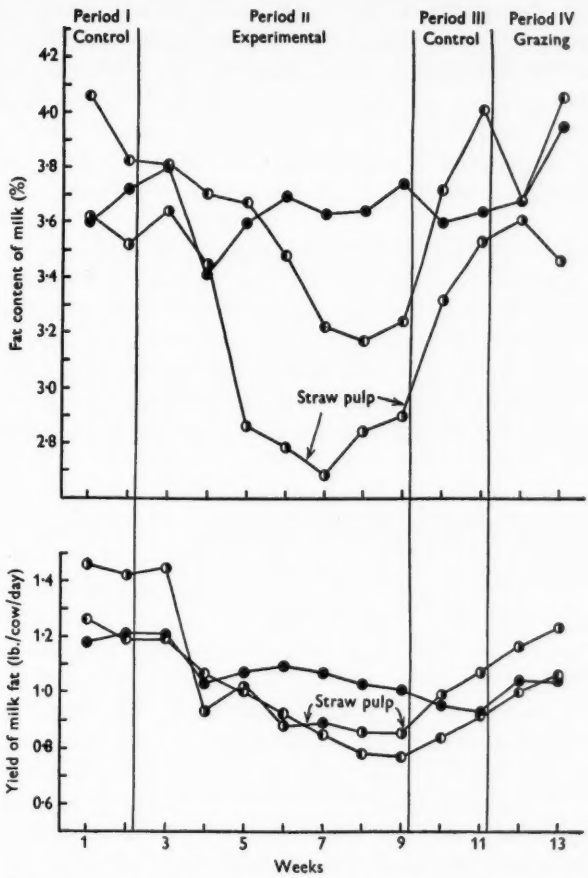

Fig. 3. The mean content and yield of milk fat, in each week of the experiment, of cows in groups A, ●; D, ◑; and E, ◐. The diets are explained in the legend to Fig. 1.

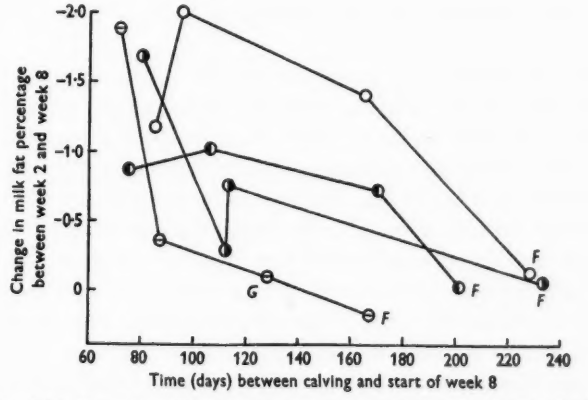

Fig. 4. Relationship of the change in milk fat content between week 2 and week 8 to the length of time the cows had been in milk. Values for individual cows receiving the various diets containing 4 lb. hay are shown, with all the values for one diet (treatment group) joined. The treatment groups were: group B, ⊖; group C, ○; group D, ◑; and group E, ◐. Values for Friesians (F) and a Guernsey (G) are marked. The diets are explained in the legend to Fig. 1.

Yield of milk fat. Also plotted in Figs. 2 and 3 are the mean yields of milk fat. In the treatment groups D and E, with allowance for small initial and final differences, this mean daily fat yield was 0·97 lb./day in weeks 4–6, compared with 1·08 in the control cows. This fall was not significant ($P > 0{\cdot}05$). Cows receiving the high protein concentrate did not fall as much as the controls in weeks 4–6, but fell to a greater extent by weeks 7–9. The fall in group C appeared to be as marked as that in groups D and E, but in group B it was less pronounced. The effect of the grazing period in increasing the fat yield was very marked.

Solids-not-fat content of the milk. The only changes in the solids-not-fat percentage to reach statistical significance were those for the change between weeks 7 and 9 in groups C and D as compared with corresponding differences for cows in group A. The changes were increases of 0·53 with the high protein ($P \doteqdot 0{\cdot}02$) and 0·43 with the low protein ($P \doteqdot 0{\cdot}05$) concentrates respectively. Groups *C* and *D* were receiving the supplementary straw pulp in the period concerned. In considering the rise in solids-not-fat it is necessary to note that at this time these were groups with the most severe depression in milk fat content.

DISCUSSION

Falls in milk fat content occurred with diets containing 4 lb. hay whether the concentrate mixture contained 11·6 or 22·3% of crude protein. The variability of the response of individual cows allows little reliance to be placed on any quantitative comparison of the relative effects of the two concentrate mixtures, but the results leave little doubt that falls in fat content are not phenomena restricted especially to diets containing either high—or low—protein concentrates, although falls may have occurred more rapidly and been somewhat greater with low protein concentrates. The large variation in the response of individual cows to treatments of this kind, was also noted by Loosli, Lucas & Maynard (5), who suspected that the response was influenced by the stage of lactation of the cows. In the present experiment the response on changing to any given treatment tended to diminish as lactation advanced, so that no appreciable falls in milk fat content were found in cows which had calved more than 170 days before the change in diet was completed. It was probably for this reason, and not because of any breed difference, that no appreciable falls were found with Friesians; both Powell (6) and Loosli *et al.* observed falls with Holsteins, and further experiments, which will be reported in this series, have shown that in early lactation the depression is at least as great in Friesians as in Shorthorns.

When receiving the control diet, a cow giving 40 lb. milk daily ate 5·7 lb. crude fibre daily. In the low-hay diet the same cow received, assuming there was no decline in milk yield, 2·2 or 1·9 lb. crude fibre with concentrate mixtures B or C respectively. The addition of 10 lb. straw pulp increased the daily crude fibre intake by only 1·0 lb. This small addition caused no recovery in milk fat. However, in later experiments, the effects of much larger additions have been investigated, and a report will shortly be published. Since the effect of the straw pulp on milk fat content was apparently negligible, it seems possible that the interesting increase in the solids-not-fat content of milk from these groups, which were those in which fat content was most depressed, may have been due to a delayed effect of the low roughage diets themselves rather than to the straw pulp. This hypothesis has also received attention and support in later experiments.

SUMMARY

1. Twenty cows were used in an experiment to compare the effect of five different diets on the fat and solids-not-fat content and yield of milk.

2. During initial and final control periods all the cows received 18 lb. hay daily and about 4 lb. of a balanced concentrate mixture (crude protein 16·5%) per 10 lb. of milk produced. In the experimental period, which lasted 7 weeks, a control group of four cows remained on this diet. Two similar groups received daily only 4 lb. hay and about 6 lb. extra concentrates; in these groups the concentrates contained 22·3% crude protein. The remaining two groups received similar treatment except that the concentrates contained only 11·6% crude protein. In addition, 10 lb. macerated delignified straw pulp (containing 1 lb. crude fibre) were given to half the cows receiving each type of concentrate. At the end of the experiment the cows were turned out to grass.

3. With the diets low in hay and high in concentrates there was a fall in the fat content of the milk, with some variability in the response of individual cows (see 4 below). With the concentrates high in protein, the fall appeared to be rather slower than with those low in protein, but by the sixth and seventh weeks of experimental treatment it was estimated to be 0·67% fat with high and 0·72% fat with low protein concentrates. The experiment showed, therefore, that serious falls in fat content may occur in cows early in lactation with diets of 4 lb. hay and concentrates containing 22·3 or 11·6% protein. The addition of a limited amount of crude fibre as straw pulp brought about no recovery in milk fat content.

4. The extent of the depression brought about by the change to diets low in hay diminished with advancing stage of lactation.

We wish to thank Mr A. S. Foot for the use of the cows, Miss H. R. Chapman and Messrs V. W. Johnson, D. G. Kemp and F. Leaver for their help on the practical aspects of the experiment, Miss R. Thorner and Messrs D. Millard and A. Wagstaff for the analyses of milk and feedingstuffs and Miss Z. D. Hosking for the computation of the statistical results.

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546. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

III. THE EFFECT OF VARIATIONS IN THE AMOUNT AND PHYSICAL STATE OF THE HAY AND A COMPARISON OF THE SHORTHORN AND FRIESIAN BREEDS

By C. C. BALCH, D. A. BALCH, S. BARTLETT, ZENA D. HOSKING, V. W. JOHNSON, S. J. ROWLAND and JILL TURNER

National Institute for Research in Dairying, University of Reading

(With 1 Figure)

Previous relevant studies abroad and at this Institute, had been concerned with the low fat content of milk from cows given less than about 6 lb. hay daily with concentrates to supply the remainder of their nutritive requirements (1). In the experiment to be reported, diets containing concentrates and 12, 8 or 4 lb. hay daily were compared, using Shorthorn cows. Since Powell (2) found that grinding hay also caused falls in milk fat content, a further group of Shorthorns received a diet containing 8 lb. ground hay, and in order to detect any breed differences a group of Friesian cows received the diet containing 4 lb. hay.

METHOD

The experiment began with sixteen Shorthorn cows. These were grouped in blocks of four, all the animals in one block having calved at about the same time. One of the blocks consisted of cows which were in rather advanced lactation and some became dry before the end of the experiment. The results for this block have, therefore, been discarded except those for the cow receiving the diet containing 4 lb. hay, which were used only for the inter-breed comparison. Four treatments (A–D) were allocated at random to the cows in each block, giving four comparable treatment groups. The main results here reported apply to three cows in each group. The four Friesian cows made up a fifth treatment group (E). The experiment lasted 14 weeks with the following programme of feeding.

Period 1 (preliminary control), weeks 1–3. All the cows received 16 lb. hay daily and concentrate mixture X (Table 1), balanced for milk production, at 4 lb./10 lb. milk produced, with small extra allowances for those that were newly calved. This mixture was the same as that used in most experiments of this series.

Period 2 (experimental treatment), weeks 4–10. The first 3 weeks were spent in gradually changing the diets of all the cows. The hay was reduced to 12, 8 or 4 lb. long hay or 8 lb. ground hay, for the various groups of Shorthorns, and to 4 lb. for the Friesians, and a second concentrate low in protein, mixture Y, was introduced to compensate for these reductions in the amount of hay. All the cows continued to receive mixture X at a little over 4 lb./10 lb. milk produced. The full treatments, as given in weeks 7–10, are summarized in Table 2.

Period 3 (final control), weeks 11–14. During 1 week the amount of hay was gradually raised to 16 lb. daily in all cows, the daily ration of mixture X continuing at 4 lb./10 lb. milk produced and mixture Y being gradually diminished and finally stopped.

Foods. The hay was of medium quality composed largely of cocksfoot with a small proportion of lucerne. The ground hay given to group D was coarsely ground in a large hammer mill fitted with its coarse sieve. Details of the concentrate mixtures are given in Table 1. The foods were sampled on alternate days and bulked for analysis by the standard methods for proximate constituents. The mean composition of the foods is given in Table 3.

Table 1. *The concentrate mixtures*

	Parts by weight	
Food	Mixture X*	Mixture Y
Barley meal	—	3
Decorticated groundnut cake	3	—
Dried brewers' grains	—	1
Dried sugar beet pulp	—	1
Flaked maize	10	3
Weatings	7	2

* Plus 2% of a commercial mineral mixture.

Table 2. *The full experimental treatments, as given in weeks* 7–10

			Amounts of food given (lb.)			
			Hay		Concentrates	
Treatment group	Breed	No. of cows	Long	Ground	Mixture X (lb./10 lb. milk)	Mixture Y
A	Shorthorn	3	12	—	4	4
B	Shorthorn	3	8	—	4	6
C	Shorthorn	3	4	—	4	8
D	Shorthorn	3	—	8	4	6
E	Friesian	4	4	—	4	8

Table 3. *Composition of hay and concentrate mixtures* (%)

Food	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Ash
Hay	83·2	10·6	1·3	34·8	30·2	6·3
Concentrate mixture X	85·4	16·1	3·5	57·6	5·0	3·2
Concentrate mixture Y	83·8	10·1	1·8	62·8	5·8	3·3

Cows. Of the twelve Shorthorns on which the main comparisons are based only one had calved more than 5 weeks before the start of the experiment.

Management. In the period between the morning and evening milkings the cows were kept in an open enclosure without herbage. At night they were housed in cowshed standings with sawdust bedding. Special partitions prevented stealing or wastage of food. The cows were milked twice daily by machine.

Milk. Details of the methods of sampling and analysing the milk have been given previously (1); only the fat content of the milk was determined. Milk samples were taken throughout the experiment with the exception of week 1.

Statistical analysis. The mean value during the initial control period (weeks 2 and 3) was used as the auxiliary variable in the analysis of covariance for the mean values over weeks 9 and 10 for treatments A–D.

For the interbreed comparison (treatments C and E) a similar analysis of covariance was carried out, but including the results for a fourth Shorthorn, from the discarded block.

RESULTS

Milk yield. The differences between the mean yields of the groups of cows, when adjusted for the initial control period values, were not significant in the treatment period ($P > 0{\cdot}1$).

The mean daily yields of milk from the cows in each treatment group are given in Fig. 1. It shows that the changes in diet did not produce marked changes in the rate of decline, but in all groups, except possibly groups D and E, the decline appeared to be somewhat faster in the period of full experimental treatment. On changing back to the control diet the decline was checked in groups A and D, while in groups B–E there were slight increases in yield. The reason for the increased decline in the experimental periods was probably that with all diets cows sometimes refused a portion of the concentrate mixture; this was especially noted in group D.

Fat content of the milk. Group weekly mean values for the fat content of the milk are also given in Fig. 1. For cows receiving 12 lb. hay daily in the experimental period (group A), there was a slow decline in milk fat content in the early weeks; in the final control period there was no appreciable rise. Unadjusted mean values for weeks 8–10 and 12–14 were 3·62 and 3·68 respectively. The mean value for week 14 was 0·23 below that for weeks 2 and 3. Since all except one of the Shorthorns had calved within 5 weeks of the start of the experiment, the whole initial decline in milk fat content in group A, and a part of the decline in other groups (see below), was probably that normally found in early lactation. It is, therefore, probable that the percentage of fat in milk from cows in group A was not affected by reducing the daily hay intake from 16 to 12 lb.

From Fig. 1 it can be seen that in all the other treatment groups the experimental diets caused declines in milk fat content. When allowance was made for differences between the treatment groups during the initial control period, adjusted values for the milk fat percentage during weeks 9 and 10 were 3·59, 2·43, 2·47 and 1·87 in groups A–D respectively. Thus by comparison with cows getting 12 lb. hay the milk fat percentage in the group receiving 8 lb. fell by 1·16 ($P \doteqdot 0{\cdot}03$) and in the group receiving 4 lb. by 1·12 ($P \doteqdot 0{\cdot}03$). Neither the difference between these values for group C compared with group B (0·04) nor the decline of 0·56 in fat percentage caused by grinding the hay (group D compared with group B) reached significance at the 10% level, but by comparison with group A the fall in group D (1·72) was significant ($P \doteqdot 0{\cdot}009$).

When adjustments were made for differences during the initial control period, the percentage of fat in milk from cows receiving 4 lb. hay daily was 2·81 for four Shorthorns (2·47 for the three used for the comparisons above) and 2·20 for four Friesians (group E). The difference between these values was not significant ($P > 0{\cdot}1$). The comparable value for the group of three Shorthorns receiving 12 lb. hay was 3·59, which gave a depression of 1·39 for the Friesians.

Yield of milk fat. The mean daily yields of milk fat in each week of the experiment are given in Fig. 1. When allowance was made for initial differences the mean daily values over weeks 9 and 10 were 1·16, 0·83, 0·82 and 0·52 lb. for groups A–D respectively. The reduction of the hay to 8 lb. (group B) or 4 lb. (group C) caused falls of 0·33 ($P \doteqdot 0{\cdot}06$) and 0·34 ($P \doteqdot 0{\cdot}06$) lb. of fat respectively as compared with group A. With 8 lb. ground hay

(group D) the fall was 0·31 lb. greater than with 8 lb. long hay ($P \doteqdot 0{\cdot}09$) and 0·64 lb. greater than with 12 lb. hay ($P \doteqdot 0{\cdot}009$). Comparable mean values for the milk fat yield of four Shorthorns and four Friesians were 0·85 and 0·74 lb. respectively; the difference was not significant ($P > 0{\cdot}1$).

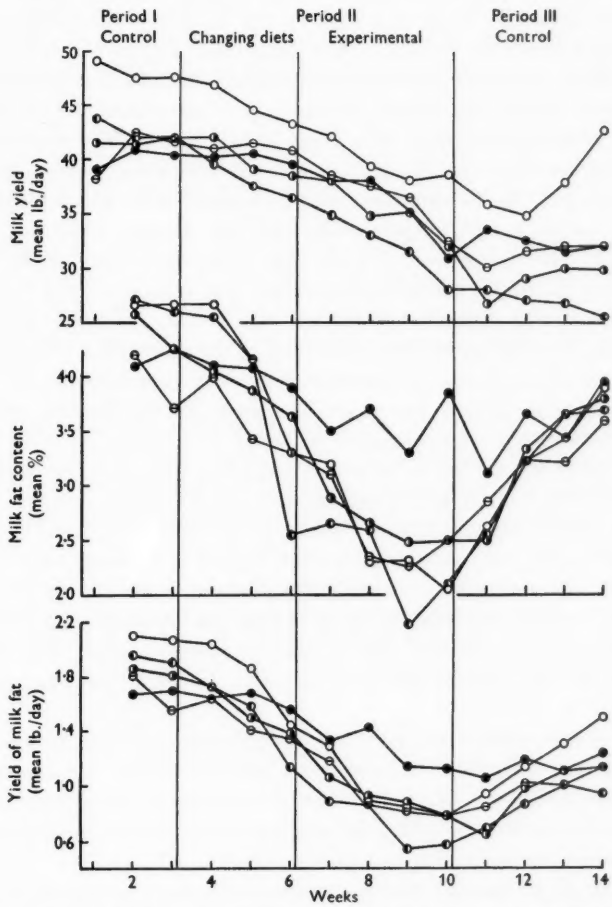

Fig. 1. The mean daily milk yield, fat content and fat yield from groups of three Shorthorn cows receiving daily in the experimental period, diet A (12 lb. hay), ●; diet B (8 lb. hay), ⊖; diet C (4 lb. hay), ◑; and diet D (8 lb. ground hay), ◐. A group of four Friesians (group E), ○, received 4 lb. hay. During the control periods all the cows received 16 lb. hay daily, and throughout the experiment the balance of their standard requirements was supplied as concentrates.

The losses of milk fat, as estimated by comparison with the amount produced by group A, were 28·4, 29·3, 55·2 and 36·2% in groups B–E respectively.

As can be seen from Fig. 1, it is likely that, for the fat percentage and fat yield, the final control period was insufficient for a full recovery, especially in groups C–E.

DISCUSSION

When the amount of hay given to a milking cow is fixed at one level and the remainder of her standard requirements for energy (starch equivalent) is supplied as concentrates, the ratio of concentrates to hay will become smaller with decreasing yields and, therefore, with advancing lactation. Thus, any effect a low roughage diet might have on, for example, fermentation in the reticulo-rumen, would probably be greater in high yielders than in low yielders or in early lactation than in late lactation. It is therefore important to emphasize that during this experiment the mean daily yields of cows in the experimental groups declined from about 40 to about 30 lb. These yields were somewhat higher than those in our previous experiments (1,3), and the cows were earlier in lactation when the experiment began. In the Shorthorn cows with yields in the above range, diet caused no appreciable decline in milk fat percentage when the amount of hay was reduced from 16 to 12 lb. daily. By comparison with the diet containing 12 lb. hay the milk fat percentage, adjusted for initial control period values, was reduced 1·16 with 8 lb. and 1·12 with 4 lb. hay; and 1·72 with 8 lb. ground hay. The further reduction of 0·56 caused by grinding the 8 lb. long hay provides confirmation of the observation of Powell (2) that the physical property of fibrousness is important for the maintenance of milk fat levels.

The fall in milk fat content was at least as great for the Friesian cows as for the Shorthorns, showing that when earlier (2) we failed to obtain a fall with Friesians the reason for this was not a difference between breeds, but more probably (and as suggested at the time) the effect of advancing lactation. Since several workers in America have found the fat content of milk from Holsteins to be lowered by diets low in roughage (see (1)), it is to be expected that Friesians would behave in a similar way. In the present experiment the fall, attributable to the dietary changes, in the fat content of milk from cows receiving 4 or 8 lb. hay daily was greater than the fall observed with cows from the same herd when given 4 or 6 lb. hay in earlier experiments (1,3): this difference was probably a result of the effect of advancing lactation, since the cows here used had calved more recently.

The results of this experiment again demonstrate that when milk fat percentages are depressed by giving diets high in concentrates and low in hay, the depressions are not due to an increased yield of milk. In fact there was a tendency towards an increased rate of decline in milk yield and the weight of fat produced by the cows represented losses varying from 28·4 to 55·2 % in the various treatment groups.

Finally it should be stressed that the concentrates given in this experiment, and in previous experiments (1,3), contained high proportions of flaked maize; later work, to be published in this series, has demonstrated that this food played a special part in the diets that depressed the fat content of milk.

SUMMARY

1. Four comparable groups of Shorthorn cows were used for an investigation of the effects of various amounts of hay, given with concentrates, on the fat content and yield of milk.

2. During the initial and final control periods all the cows received 16 lb. hay daily and about 4 lb. of a balanced concentrate mixture (flaked maize 50 %, weatings 35 % and decorticated groundnut cake 15 %) per 10 lb. of milk produced. During the experimental

treatment period of 7 weeks the groups received 12, 8 or 4 lb. hay, or 8 lb. coarsely ground hay, per cow daily respectively, with sufficient of a low protein concentrate to compensate for the reduction in hay, and the balanced mixture as in the control periods.

3. The change from 16 to 12 lb. hay did not affect the fat content of the milk, a small decline being attributable to the advance in stage of lactation, but the mean values for the last 2 weeks of treatment, adjusted for the differences in the initial control period, showed that the milk fat percentage for the groups receiving 8 and 4 lb. hay and 8 lb. ground hay had fallen by 1·16, 1·12 and 1·72 respectively by comparison with that for the 12 lb. hay group.

4. A group of Friesian cows was included in the experiment, and received 4 lb. hay during the treatment period. The fall in the fat content of their milk was greater, but not significantly different from, that observed with the corresponding group of Shorthorn cows.

5. In the treatment period the milk yields tended to decline more rapidly than in the control periods, and this taken in conjunction with the falls in fat content was reflected in considerable falls in the yield of milk fat, ranging from 28·4 to 55·2% with diets containing less than 12 lb. hay. Both the fat percentage and fat yields recovered in the final control period.

We wish to thank Mr A. S. Foot for the use of the cows, Mr C. P. Cox for his guidance on the statistical treatment of the results, Messrs D. G. Kemp and F. Leaver for their care of the animals, Mrs R. Winder and Mr A. Wagstaff who carried out the numerous analyses of the milk, and Misses B. K. Allchorne and K. Rhymes for the statistical computations.

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547. A RECORDING TYMPANOMETER FOR THE MEASUREMENT OF INTRAMAMMARY PRESSURE IN THE COW

By D. S. M. PHILLIPS

Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand

(With 3 Figures)

INTRODUCTION

The problem of the measurement of the intramammary pressure in the cow is one of considerable importance in the study of milk secretion, since changes in pressure are the only easily observed manifestations of the complex changes which take place in the udder during the process of milk ejection or 'let-down'. Further, during the subsequent removal of milk from the gland, the milk pressure in the lower cistern and teats is an important factor in determining the rate of milking. A full understanding of pressure changes is therefore of considerable practical importance. For such a measurement to be of value, the premeasurement preparation and the procedure employed in attaching the measuring element, must be simple. Any interference with the gland prior to the normal stimulus producing 'let-down' will usually produce undesired effects, by inhibiting the let-down reflex or producing a partial stimulus before the normal stimulus is applied. The ideal is a portable instrument which can be brought into the milking stall immediately after the normal stimulus has been given, and applied to the cow in such a way that no discomfort or unusual sensation of any kind is produced.

The measuring technique most suitable to these conditions is the 'tympanometer' principle described by Kleiber(1). This technique for measuring the fluid pressure inside a thin-walled elastic container involves applying a measured force to a small portion of the distended surface, sufficient to flatten the area of contact without causing an indentation. Under these conditions the stresses acting in the plane of the contact surface are at right angles to the direction of external thrust and, therefore, do not contribute to the forces on the pressure plate. This is only true if the surface of contact is smooth and the wall thickness of the elastic container is not great. The external thrust applied to the pressure plate is then equal and opposite to the fluid pressure acting on the inside of the container. This method makes it possible to measure the intramammary pressure without interfering unduly with the udder.

DESCRIPTION

The instrument was required to measure and record static, as well as changing pressures, in the range 0–30 in. of water.

The complete apparatus consists of two main units: (1) a tympanometer measuring unit, and (2) a continuous recording attachment.

(1) *Tympanometer measuring unit*

This was first developed and used to make individual readings which were indicated on a scale. In an improved form the tympanometer was coupled to a recorder in order to

give an accurate graph of the pressure changes being studied. A sectional view of the tympanometer unit is shown on the left of Fig. 1. The unit is similar in construction to the instrument described by Kleiber (1).

The body of this unit carries a screw *A*, which is used to adjust the tension in the spring *B*. This spring engages the end of a flat-ended spindle *C*, supported between two diaphragms of thin P.V.C. sheet. The front plate of the outer diaphragm *D* is placed in firm contact with the udder surface. To measure the pressure, the screw *A* is first screwed out until the front diaphragm is forced in by the milk pressure, so causing the contact *E* to touch the front plate of the spindle *C*, lighting the lamp *F*. The screw *A* is then screwed in to the point at which the lamp is extinguished. The front plate *D* is then parallel with the front ring of the unit, and the internal milk pressure in the udder is just balanced by the spring tension applied to the plate *D*.

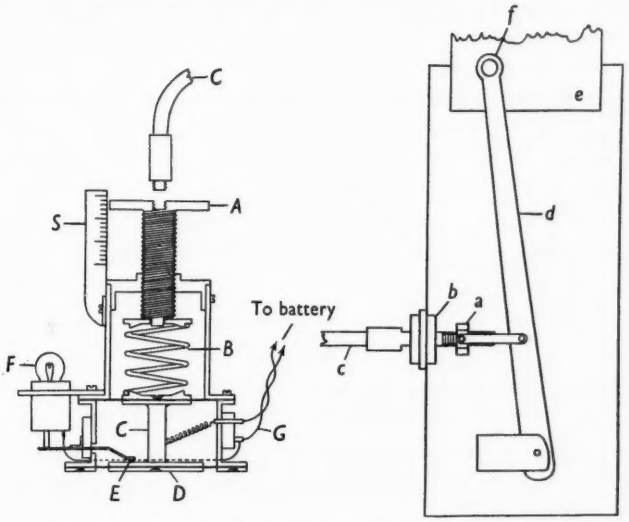

Fig. 1. A cross-section drawing of the tympanometer unit (left), and its associated recorder (right).

This technique is applied for individual readings of pressure, and the readings are made from a scale *S*, calibrated at $\frac{1}{10}$ turn intervals.

In the instrument described, two important modifications of Kleiber's design are employed. A contact system is used to determine the point at which the front diaphragm and ring are parallel, and a clear visual indication is obtained by means of a small lamp. The use of two diaphragms to support the moving parts eliminates friction and provides a steady movement even though the changes in force are extremely small.

(2) *Attachment for continuous recording*

Since individual readings are of limited use in following rapidly changing pressures, a method of continuously recording the readings is very desirable. This was achieved in a simple manner, using the attachments shown on the right of Fig. 1. A length of about 30 in. of flexible drive cable is fitted to screw *A*, and to a fitting *b* on the recorder unit. When the screw *A* is rotated the cable causes the threaded portion of fitting *b* to turn inside the fixed threaded collar *a*. This in turn is attached through a pivoted link to an

arm *d* (5 in. long) carrying a ball-pen *f*. This pen makes a record on a moving paper chart *e*, driven by a small clockwork motor. With this attachment, all the adjustments made to *A* are recorded on the chart *e*.

APPLICATION

Since the pressure measurements generally are to be carried out in the milking stall under rather awkward conditions, a portable apparatus is desirable. The present apparatus is ideal in this respect. As the recording unit can be strapped to the waist of the operator, his hands are free to hold the measuring unit at any desired place on the udder. To follow a pressure rise the instrument is adjusted to the point where the lamp is extinguished. When the pressure increases the lamp relights and it becomes necessary to adjust the screw *A* repeatedly to keep the lamp at the extinguishing point. Each adjustment is recorded on the moving chart as a small step. If care is taken a typical record will consist of fifty or more such steps and is almost a continuous line (see Fig. 2).

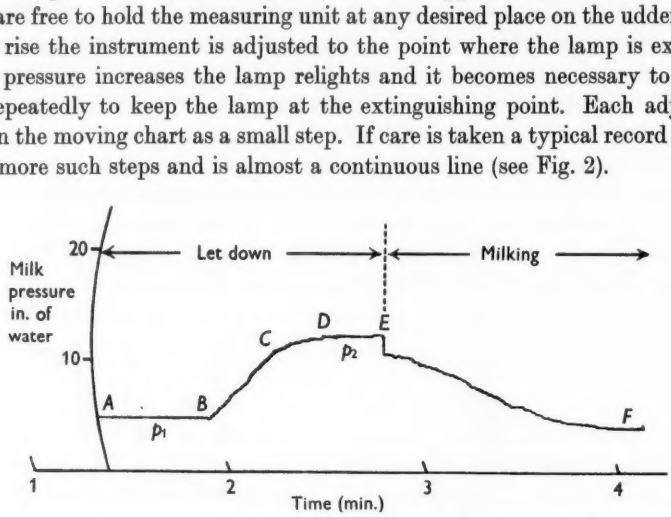

Fig. 2. A typical record of the change in pressure in the milk cistern of the cow mammary gland during the milk-ejection process and subsequent milking.

To follow a pressure decrease the procedure is similar except that the instrument is first adjusted to the point where the lamp just lights and is subsequently re-adjusted each time the lamp extinguishes.

CALIBRATION

The instrument was calibrated, using an artificial pressure system consisting of a rubber diaphragm stretched over a container. This was filled with water and connected to a movable reservoir, which was used to adjust the pressure on the inside of the diaphragm. A reading of the pressure on the diaphragm was made with the tympanometer, and the corresponding head of water in the reservoir was measured.

A further practical check was made on an actual mammary gland. The pressure rise in the milk cistern of the gland was followed by means of a teat canula connected to a U-tube containing milk from the gland as the indicating liquid. At the same time a record of the pressure rise was made using the tympanometer. The two curves obtained showed very good agreement.

TYPICAL RECORDS

A typical record of the pressure changes in the milk cistern of a cow's udder during the 'let-down' process and subsequent milking is shown in Fig. 2.

The instrument was applied to the wall of the udder before let-down took place. At point *A* adjustment to the initial pressure p_1 was completed and during the interval *A*–*B*

the pressure remained constant. At *B* milk transfer commenced and the pressure increased rapidly to point *C*, and thereafter increased more slowly to a pressure p_2 at *D*. After point *D* the pressure remained constant indicating that milk transfer in the udder had ceased. At point *E* the milking machine was applied and the milk pressure declined slowly as milk was removed from the udder, reaching a low level at *F* where milking was almost complete.

Such records can provide accurate timing of the processes controlling let-down as well as accurate measurements of pressure levels and the rate of pressure change.

The application of the instrument described is limited by the fact that it is unsuitable for the measurement of fluctuating pressures. A modification, using two sets of contacts and two lamps, was tried which gave accurate bi-directional recordings, but some practice was necessary before good results could be obtained. However, in the work for which the instrument was designed, the pressure changes are continuous and the limitation mentioned above no disadvantage.

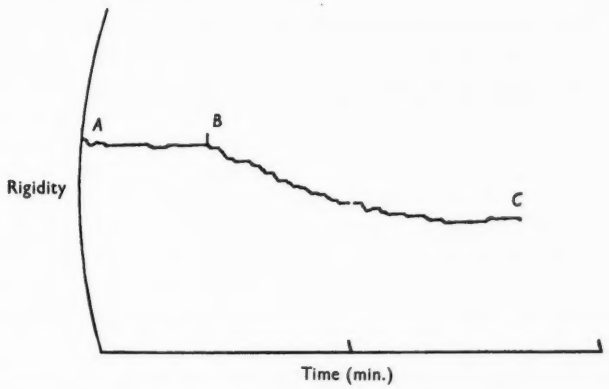

Fig. 3. A typical record of the change in 'rigidity' of the secretory tissue of the cow mammary gland during the milk-ejection process.

FURTHER DEVELOPMENTS

This instrument can be used also as a 'penetrometer' type of 'pressure meter'. In this application, the rigidity of a surface may be determined by measuring the force required to press a small rounded boss a fixed depth into the surface. This type of measurement was carried out on the secretory portion of the mammary gland, where the rigidity of the secreting tissue decreases as the milk is forced out, this action being complementary to the rise in fluid pressure in the lower cistern. Such a record is shown in Fig. 3.

At the point *A* the initial 'rigidity' at the point of measurement on the gland is constant before 'let-down' occurs. At *B* the transfer of milk commences and the 'rigidity' declines to a new level *C* indicating that milk transfer has ceased.

CONCLUSION

The instrument described is sufficiently accurate for the purposes intended, and pressure records can be produced which show a considerable amount of detail. The apparatus has the great advantage of simplicity and ease of manipulation. As complicated equipment

cannot easily be set up in the milking stalls, the portable nature of the equipment is a great advantage.

SUMMARY

A manually operated tympanometer pressure recorder of simple construction designed for recording the changes in pressure in the lower cistern of the mammary gland of the cow during let-down and milking is described. The instrument makes possible the measurement of the internal milk pressure without the need for direct access to the milk cistern. The measurements are made with a tympanometer unit which is held firmly against the outside wall of the udder.

The author is indebted to Mr W. G. Whittlestone for suggesting the use of the tympanometer principle and to Mr K. C. Mills, formerly of this Station, for his skill and co-operation in the construction of the equipment.

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548. INTRAMAMMARY PRESSURE CHANGES IN THE LACTATING SOW

II. THE EFFECTS OF VASOPRESSIN AND ACETYLCHOLINE

By W. G. WHITTLESTONE

Ruakura Animal Research Station, Department of Agriculture, New Zealand

(With 3 Figures)

In Part I of these studies, the effects of oxytocin and whole pituitary extract on the intramammary pressure of the lactating sow were recorded and the recording apparatus was described in detail. The present studies were carried out with the same apparatus and injection technique. Because of the current interest in the action of vasopressin on uterine muscle and the establishment of genuine oxytocic activity in pure vasopressin preparations (1), it seemed of interest to examine in more detail the action of this hormone on the mammary gland. In earlier studies (2,3), the present writer was led to believe that vasopressin has milk-ejecting properties of its own, and highly purified vasopressin was found to have one unit of milk-ejecting activity (compared with standard oxytocin) to five units of pressor activity. The method of estimation depended on measuring the duration of milk ejection in the lactating sow. It seemed possible that similar ejection times could be associated with different reactions to the hormones injected. Hence, by using pressure-recording apparatus it was hoped that differences in reaction might be detected.

The part played by vasopressin in the milk-ejection process is by no means clear. Peeters (4), Cross (5), Kalliala & Karvonen (6) and Kalliala, Karvonen & Leppanen (7) have shown that in the cow, the rabbit, the human and the dog the milk-ejection reflex is associated with the release of the anti-diuretic hormone. It seems that this component of the posterior pituitary complex is associated with milk ejection in that the two phenomena, milk ejection and anti-diuresis, occur together. Does this suggest that the whole complex is released on stimulation so that both oxytocin and vasopressin act on the mammary gland, or is vasopressin the milk-ejecting hormone? The fact that oxytocin has much greater ejecting activity than vasopressin does not necessarily prove that it is in fact the hormone involved. It is known that vasopressin will eject milk in the sow, the rabbit (8), and the cow (9). Normal ejection is associated with the characteristic anti-diuretic action of the pressor fraction so that the evidence for its involvement in ejection is good. There is no direct evidence for the view that oxytocin is the sole milk-ejecting hormone (10), though the present writter (11) has drawn attention to the fact that in the sow prepartum uterine contractions are associated with milk ejection and post-partum uterine contractions following suckling are well known (12).

The following experiments compare the effects of vasopressin and acetylcholine with oxytocin in their action on the sow mammary gland as determined by changes in intramammary pressure.

The effects of vasopressin

Fig. 1 shows the effect of injecting 2·5 i.u. of commercial vasopressin compared with the effect of 0·5 i.u. of commercial oxytocin. There is no apparent difference between the responses. The second and third graphs show small secondary changes in pressure which have been described earlier (11).

Fig. 2 shows a series of pressure changes associated with alternating injections of oxytocin and vasopressin. Table 1 sets out the order of the doses, the time from injection to the commencement of ejection, the duration of ejection as judged by hand milking and

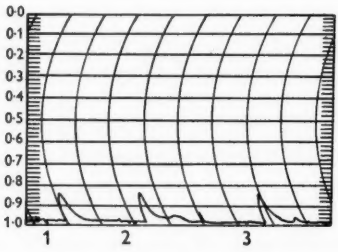

Fig. 1. Intramammary pressure rise due to 2·5 i.u. of vasopressin (graph 2) compared with 0·5 i.u. oxytocin (graphs 1 and 3). Scale: time: 1 division = 30 sec., pressure: 1 large division = 15 mm. Hg.

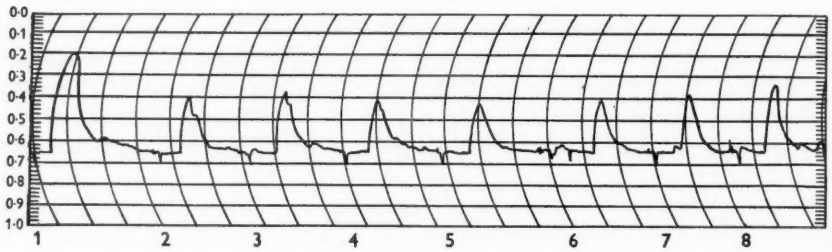

Fig. 2. Intramammary pressure changes caused by alternating injections of oxytocin and vasopressin (see Table 1 for further details). Scale: time: 1 division = 30 sec.; pressure: 1 large division = 5·6 mm. Hg.

the pressure rise. The first injection of 0·5 i.u. of oxytocin gave a larger amplitude than subsequent equivalent doses. This appears to be characteristic of such a series of responses. The second injection of 2·5 i.u. of vasopressin gave a response with a suggestion of a diphasic character (11). The third injection of 0·5 i.u. of oxytocin gave an almost identical response in terms of the shape of the graph. The fourth graph corresponding to 2·5 i.u. of vasopressin was again of the same character. The graphs 5 to 8 representing responses to 0·25 i.u. of oxytocin, 1·25 i.u. of vasopressin, and 0·25 i.u. of oxytocin, are again very similar. The last graph, number 8, gives the response to a mixture of 0·25 i.u. of oxytocin and 1·25 i.u. of vasopressin. The augmented response suggests that the two components function additively.

An examination of the foregoing data shows that no obvious difference may be detected between the action of oxytocin and that of vasopressin on the type of response given by the lactating sow when measured as a pressure change.

Table 1. *The effect of various doses of oxytocin and vasopressin on the ejection of milk in the sow (see also Fig. 2)*

Dose (i.u.)	Hormone	Time from injection to ejection (sec.)	Duration of ejection (sec.)	Pressure rise (mm. Hg.)	Graph number in Fig. 2
0·5	Oxytocin	16	44	25·0	1
2·5	Vasopressin	20	42	14·5	2
0·5	Oxytocin	28	35	15·5	3
2·5	Vasopressin	25	31	13·5	4
0·25	Oxytocin	28	—	12·5	5
1·25	Vasopressin	22	32	13·0	6
0·25	Oxytocin	26	23	14·5	7
0·25*	Oxytocin	28	41	17·0	8
1·25	Vasopressin				

* In this last experiment the oxytocin and vasopressin were given together.

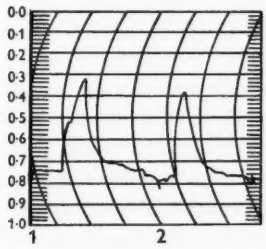

Fig. 3. Intramammary pressure changes caused by acetylcholine (graph 2) and by oxytocin (graph 1). Scale: time: 1 division = 30 sec.; pressure: 1 large division = 5·0 mm. Hg.

The effects of acetylcholine

In an earlier paper the milk-ejecting activity of acetylcholine in the lactating sow was described (13). It was of interest to know whether the pressure response is similar to that produced by oxytocin. Fig. 3 shows the response of a sow to 0·2 g. of acetylcholine injected intravenously. The first graph shows the response to 0·5 i.u. of oxytocin, the second to acetylcholine. The pressure change appears to be of the same form as that produced by oxytocin. Sows were found to be very variable in their reaction to acetylcholine. Some gave no response to 0·2 g. doses in terms of intrammary pressure changes, but showed other reactions such as excessive salivation. One sow which came from a family with a history of heart weakness died as a result of injecting 0·2 g. acetylcholine.

It is clear, however, that the sow's mammary myoepithelium reacts in the same manner to acetylcholine as it does to oxytocin.

The effects of histamine

Because of its action on uterine muscle and hence its confusing effects on oxytocin assays, it was thought useful to examine the effect of histamine on the sow mammary gland. The possibility that certain earlier milk-ejection results (14) could have been due to histamine as an impurity in the extracts could not be entirely excluded because of the similarity in reaction of uterine muscle and mammary myoepithelium in several respects.

The sows chosen for the experiment were Large Whites which, because of their unpigmented skins, showed clearly the effects of vasodilation. An intravenous dose of

0·2 mg. histamine produced no effect within the mammary gland, but produced a visible 'blush' which extended over the entire mammary gland area. The injection was increased to 0·4 mg. producing even more marked vasodilation but still having no effect on milk ejection. An injection of 0·5 i.u. of oxytocin followed the second histamine injection in 72 sec. This produced a normal ejection response.

It would seem that an amount of histamine which is capable of producing a marked expansion of the skin blood vessels will not produce any measurable effect on the sow mammary gland. No posterior pituitary extracts assayed on a white sow have been observed to produce detectable vasodilation, so that it would seem safe to conclude that the responses recorded in earlier publications are due to the posterior lobe hormones and not to histamine.

DISCUSSION

The intramammary pressure changes which are produced in the lactating sow by oxytocin, vasopressin or acetylcholine are apparently of the same kind. Despite the associated pressor effects of vasopressin and the range of effects produced by acetylcholine, the pressure change curves are similar to those produced by oxytocin, which is generally regarded as having fewer side effects than these other substances. Histamine appears to have no effect on the mammary gland at the concentrations used.

There are some differences between the reactions of the mammary gland of the intact sow and those of the perfused bovine udder described by Petersen (15) with respect to the substances tested so far. Petersen reported that histamine gave a partial evacuation of the bovine gland in the range 0·5–10 mg., while carbaminoyl choline had no effect. Earlier experiments on the sow (13) showed this latter substance to cause milk ejection in the same way as acetylcholine. Petersen showed that adrenaline produced partial ejection, and intramammary pressure measurements on the sow at this station have shown no changes with doses of up to 1000 μg. adrenaline. The reasons for these differences are not immediately obvious and indicate the need for further work on the sow. The sow has been chosen for these studies because of the simple construction of her mammary gland. Future studies will include the more complex cow gland.

It would seem that the two posterior pituitary hormones have a similar action on the sow mammary gland, and it is probable that both are released by the suckling stimulus.

SUMMARY

Measurements of intramammary pressure changes in the sow following intravenous injection have shown that the milk-ejecting action of vasopressin and acetylcholine is similar to that of oxytocin. Doses up to 0·4 mg. of histamine produced no measurable effects within the mammary gland.

The writer is indebted to Mr D. M. Smith for the use of the sows and to Mr E. D. O'Reilly for technical assistance.

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612.664.3:636.4

549. INTRAMAMMARY PRESSURE CHANGES IN THE LACTATING SOW

III. THE EFFECTS OF LEVEL OF DOSE OF OXYTOCIN AND THE INFLUENCE OF RATE OF INJECTION

By W. G. WHITTLESTONE

Ruakura Animal Research Station, Department of Agriculture, New Zealand

(With 7 Figures)

Earlier papers(1,2,3) have described a technique for recording intramammary pressure changes in the lactating sow and have shown some of the effects of oxytocin, vasopressin, acetylcholine, adrenaline and histamine. This present study deals with two groups of effects: (*a*) the nature of the intramammary pressure changes which occur at low concentrations of oxytocin, and (*b*) the effects of varying rates of injection with both small and large concentrations of oxytocin. In earlier work evidence was produced for the view that a peak of concentration is required to trigger the contraction of the mammary myoepithelium. The first part of this study deals with effects at or about the threshhold level and aims at determining the order of amount of oxytocin required to produce a measurable reaction of the sow's mammary gland. Both the concentration of oxytocin and the rate of injection were varied in the experiments.

(*a*) *The effects of low concentrations of oxytocin*

Experiment 1

The sow used in this experiment was injected at 3 min. intervals, a siliconed needle being kept in one place in an ear vein throughout the series of injections. When an injection time is not specified the time required was that normally taken to force 0·5 ml. of solution through a fine needle, and was of the order of 2 sec. or less. Recorded injection times indicate the period from the beginning of the injection until the full dose was delivered.

Fig. 1 shows the reactions of a sow to a series of injections of oxytocin ranging from 0·03 to 0·1 i.u. A dose of 0·05 i.u. evoked a positive response, whereas 0·03 i.u. gave extremely little. When a 0·06 i.u. dose was given in 6 sec. a marked pressure rise occurred. The same dose given in 15 sec. produced no response. Given in 9 sec. a reduced but positive pressure rise occurred, while at 11·5 sec. only a very small reaction appeared.

Experiment 2

The same sow was used under the same conditions, injections being given at 3 min. intervals. Fig. 2 illustrates the results. Again 0·05 i.u. produced a positive response. The reactions to varying rates of injection were not consistent in the range 6–10 sec. Above 10 sec. no response occurred, and between 6 and 10 sec. the reaction was erratic.

Experiment 3

Another sow was used for this experiment. Her reactions are given in Fig. 3. She had rather small, tight glands so that bodily movements showed up clearly on the pressure

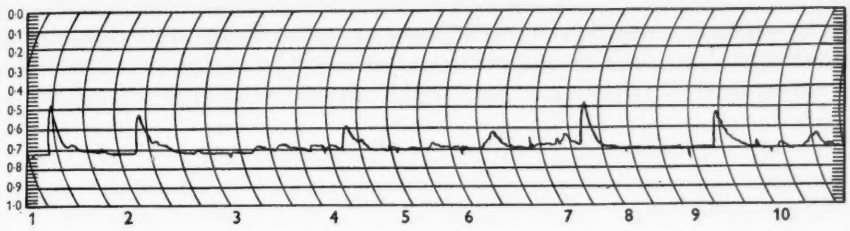

Fig. 1. Intramammary pressure changes caused by injecting various doses of oxytocin. The doses and the rates of injection are given below:

No.	Injection (i.u.)	No.	Injection (i.u.)
1	0·10*	6	0·03
2	0·05	7	0·06 in 6 sec.
3	0·04	8	0·06 in 15 sec.
4	0·04	9	0·06 in 9 sec.
5	0·03	10	0·06 in 11·5 sec.

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

* Where no rate of injection is given the time taken was less than 2 sec.

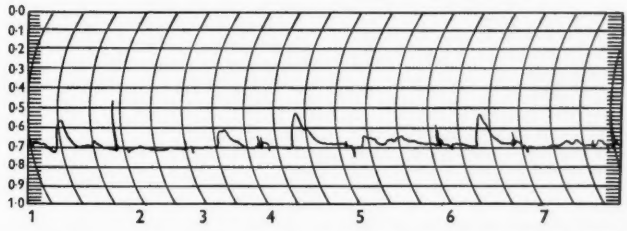

Fig. 2. Intramammary pressure changes caused by the doses of oxytocin and injection rates shown below:

No.	Injection (i.u.)	Time (sec.)
1	0·05	Less than 2
2	0·06	6
3	0·06	3
4	0·06	5
5	0·06	8
6	0·06	10
7	0·06	15

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

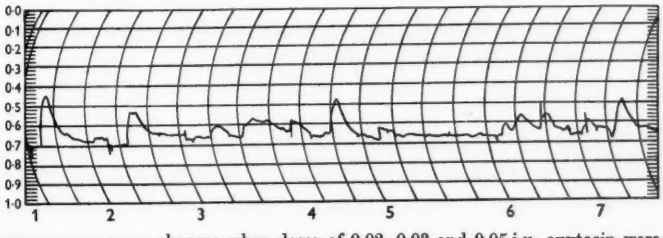

Fig. 3. Intramammary pressure changes when doses of 0·02, 0·03 and 0·05 i.u. oxytocin were given each in under 2 sec.:

No.	Injection (i.u.)	No.	Injection (i.u.)
1	0·05	5	0·02
2	0·03	6	0·02
3	0·02	7	0·03
4	0·03		

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

record. Again 0·05 i.u. of oxytocin gave a definite rise in pressure; 0·03 i.u. also consistently produced a reaction. When the dose was reduced to 0·02 the response was dubious in two cases and definitely zero in one. All injections were given in 2 sec. or less. The minimum dose producing a clear positive reaction when injected quickly was 0·03 i.u.

Experiment 4

The sow used in Exps. 1 and 2 was used again for Exp. 4. All injections were given quickly at 3 min. intervals. A siliconed needle was left in position except that after the seventh injection the needle had to be moved to a nearby site. Fig. 4 shows the pressure changes. Again 0·05 i.u. produced a marked pressure change, 0·04 i.u. gave no reaction after two injections and a dubious change after one. A dose of 0·045 gave a definite response.

Note. The weights of the sows used in Exps. 1–4 were: Exps. 1, 2, and 4, 387 lb.; Exp. 3, 395 lb.

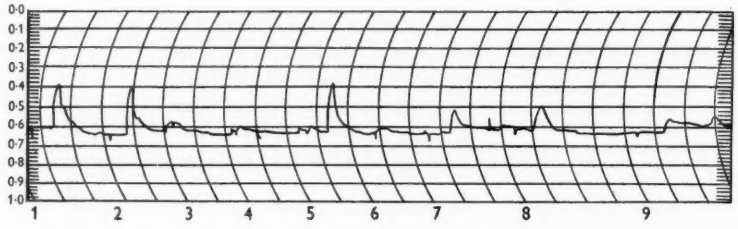

Fig. 4. Intramammary pressure changes resulting from injecting 0·03 to 0·05 i.u. oxytocin in less than 2 sec.:

No.	Injection (i.u.)	No.	Injection (i.u.)
1	0·05	6	0·04
2	0·05	7	0·05
3	0·03	8	0·045
4	0·04	9	0·04
5	0·05	10	0·05

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

(*b*) *The effects of large amounts of oxytocin*

Earlier experiments (2) showed that doses of oxytocin considerably above the minimum required to give a reaction occasionally produced a biphasic pressure graph. The following experiments continued the investigation of this point.

Experiment 5

A dose of 0·8 i.u. of oxytocin was injected in 8 sec. in two tests and in 1·5 sec. in two further tests. The results are shown in Fig. 5. The two slow injections produced definitely biphasic responses while one of the fast injection curves was distinctly monophasic and the other showed signs of a small second pressure rise.

Experiment 6

The results of injecting 1·0 i.u. of oxytocin in different times are recorded in Fig. 6. The injection times were 0·5 sec. (0·1 ml. of solution); 15 sec. (1·0 ml. of solution); 0·2 sec. (0·1 ml. of solution). The different volumes of solution were used to enable the injection

time to be varied over a wide range. Multiple responses were obtained in all cases. The fourth graph in this figure shows the reaction to 5·0 i.u. injected in 0·5 sec. This large dose, which is well above physiological level, produced a flow of milk lasting about 250 sec. There is some sort of periodicity about the pressure graph, but it is difficult to measure the interval between pressure waves. In graphs 1–3 the periods between the waves, measured from the peaks of each rise, were 18, 12, 10, 18, 18, 14 sec. The fourth graph, while showing what happens following a very large injection, supplies little reliable information on the periodicity of the ejection mechanism. The following periods between peaks may be recorded, but owing to the erratic nature of the curve it is possible that some contractile cycles were missed: 12, 12, 18, 18, 28 sec.

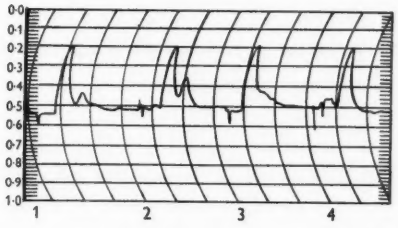

Fig. 5. Intramammary pressure changes resulting from 0·8 i.u. oxytocin and the injection times shown below:

No.	Injection (i.u.)	Time (sec.)
1	0·8	8·0
2	0·8	8·0
3	0·8	1·5
4	0·8	1·5

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

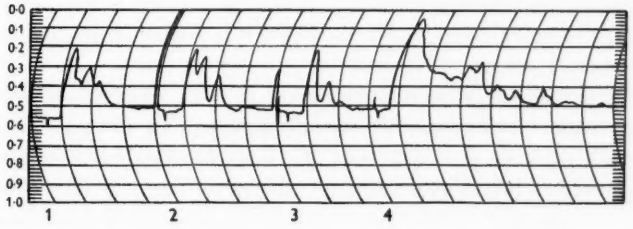

Fig. 6. Intramammary pressure changes resulting from very large doses of oxytocin:

No.	Injection (i.u.)	Time (sec.)	Vol. (ml.)
1	1·0	0·5	0·1
2	1·0	15·0	1·0
3	1·0	2·0	0·1
4	5·0	0·5	—

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

Experiment 7

Another sow was used for this experiment. This animal had a tight mammary gland and tended to move about in the stall. Hence erratic pressure changes occurred from time to time (Fig. 7). These changes were associated with the sow raising her head above the stall gate and were readily distinguished from genuine ejection changes. In this experiment 0·8 i.u. of oxytocin was injected in 8, 1·5 and 22 sec. respectively. The first two

ejection graphs show unmistakable biphasic responses with periods of 18 sec. The third graph, which has a smaller amplitude than the previous two, is of a different form, though multiple ejection would seem to be the cause of the wave structure.

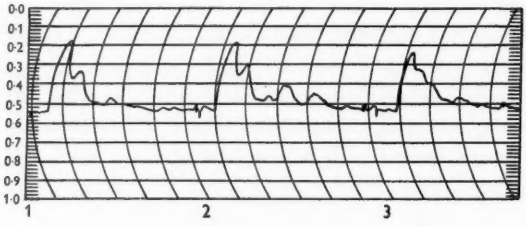

Fig. 7. Intramammary pressure changes when 0·8 i.u. oxytocin was given at the injection rates shown below:

No.	Injection (i.u.)	Time (sec.)
1	0·8	8·0
2	0·8	1·5
3	0·8	22·0

Scale: time divisions = 30 sec. Pressure, full scale = 50 mm. Hg.

Discussion on Exps. 5–7

In the first experiment of this series (no. 5) the slow injection of 0·8 i.u. of oxytocin resulted in biphasic pressure graphs with periods of 18 sec., while corresponding rapid injections produced simple curves. On the other hand, in Exps. 6 and 7 all injections produced multiple pressure curves. The reason for the differences is not understood. One point, however, may be involved. The ultimate effective rate of injection is controlled by the rate of flow of the blood through the small ear veins. Occasionally a vein is seen to distend and release its contents gradually. While this did not occur as far as could be detected during the experiments under discussion it is possible that such an effect could have influenced the results.

In the earlier experiments on intramammary pressure changes secondary pulses of pressure were recorded 22–25 sec. after the initial pulse. This effect does not occur when the dose is small or when it is large and the main pressure curve eclipses any small changes. If these small pulses were due to a return of the hormone after one circuit of the circulatory system, the pulses in the multiple graphs of Exps. 5–7 would seem, because of the period between them, to be due to another cause. In the light of the evidence so far obtained the contractile waves would appear to be due to the inherent response of mammary myoepithelium to oxytocin at concentrations above the minimum needed to start contraction. It is unlikely that the periodic contractile characteristics of the sow's myoepithelium are peculiar to this animal. Investigations into this aspect of milk ejection in other domestic mammals are planned. In particular the ejection process in the cow will be studied in the light of the foregoing observations.

SUMMARY

The injection of small amounts of oxytocin into the lactating sow has shown that the minimum quantity required to evoke a response in a normal adult animal is of the order of 0·05 i.u. If the injection is prolonged, the dose must be increased to produce a response.

Large doses (0·8–1·0 i.u.) given at widely different rates produce multiple ejection pressure graphs with two or more peaks.

Mr D. M. Smith made the sows available for these studies and the injections were carried out by Mr E. D. O'Reilly. The writer is grateful to both for their help.

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550. COLOUR CHANGES IN HEATED AND UNHEATED MILK

I. THE BROWNING OF MILK ON HEATING

By H. BURTON

National Institute for Research in Dairying, University of Reading

(With 11 Figures)

INTRODUCTION

The browning which occurs when milk is heated at temperatures above 100° C. is a familiar phenomenon. It is of considerable importance in the commercial production of so-called 'sterilized' milk since, as has been shown in a recent paper (1), the proportion of spoilage is to a large extent governed by the brownness that can be tolerated in the processed milk.

The browning of milk is now generally considered to be an example of a general class of condensation reactions between amino-acids and aldehydes which produce dark coloured pigments as end products (2). With milk, the reacting amino and aldehyde groups are provided by the proteins and lactose present. Other workers (3) have held that the reaction is a caramelization of the lactose, followed by adsorption of the caramelized material on the protein. It is generally agreed, however, that the brown coloration is so closely associated with the protein that only by rendering the protein itself soluble can the colour be released into solution for measurement by conventional colorimetric methods.

Several methods for measurement of the degree of brownness have been suggested and used in investigations of the reaction. Doob, Willmann & Sharp (4) used a method of alkaline extraction to obtain the pigment in clear solution. A more satisfactory way of obtaining the coloured material in solution for colorimetric estimation is the method of trypsin digestion of the protein used by Choi, Koncus, O'Malley & Fairbanks (5).

Methods of colour measurement not involving chemical procedures would be of practical value for routine examination and process control. Webb & Holm (6) used the Munsell colour system for work on evaporated milk. In this system the colour to be measured is compared with a synthetic colour consisting of a mixture of the primary colours and of neutral greys, the mixing being carried out by means of spinning disks. The Lovibond Universal Tintometer, using similar principles, was tried in the early stages of the present work, but was found not to be sufficiently sensitive for the measurement of slight colour changes.

Since the colour of an opaque medium is in fact a characteristic spectral distribution of light reflected from it, the spectral distribution of reflectance is a statement of the colour of the medium. This method of colour determination was used by Nelson (7, 8) in work on evaporated milk. The preliminary experiments reported by Burton, Akam, Thiel, Grinsted & Clegg (1) showed that the method was equally suitable for use with sterilized milk.

The purpose of this paper is to show how reflectance methods can be used in the investigation of the browning reaction in milk, and to present information on the characteristics of the reaction obtained by these methods.

EXPERIMENTAL METHODS

Methods of reflectance measurement

Two different instruments were used for reflectance measurements, a Beckman spectrophotometer with reflectance attachment, and an EEL reflectance spectrophotometer (Messrs Evans Electroselenium Ltd.).

The Beckman spectrophotometer consisted of the standard quartz monochromator and photocell housing, with a reflectance attachment made at the National Institute for Research in Dairying using optical components provided by Messrs Unicam Ltd. The layout of this unit is shown diagrammatically in Fig. 1. The milk was contained in a shallow circular dish having a depth of at least 1 cm. so that the measurements were not affected by light reflected from the bottom of the container. As the reflectance at the free milk surface was measured, this surface had to be about 2 mm. below the level of the top of the dish to prevent fouling of the bottom of the reflectance attachment. The reflectance obtained is affected by the milk level, so that the same volume of milk was placed in the dish at each measurement.

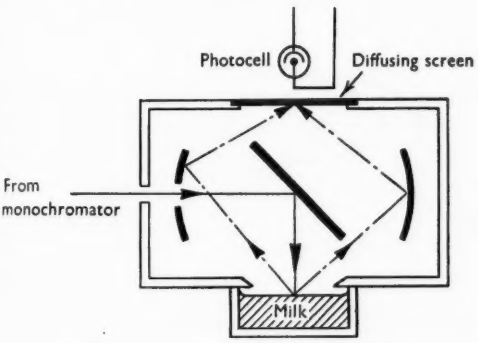

Fig. 1

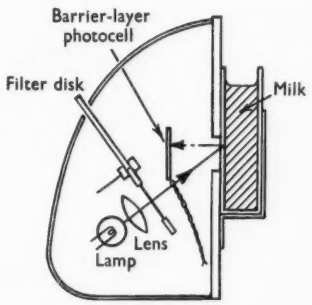

Fig. 2

Fig. 1. Arrangement of reflectance attachment for Beckman spectrophotometer. —→—, incident light; – –→– –, light reflected diffusely.

Fig. 2. Arrangement of 'EEL' reflectance head. —→—, incident light; – –→– –, light reflected diffusely.

This system is suitable for precise work with monochromatic incident light over a wide range of wave-lengths. It is not, however, convenient for large numbers of samples where less precise results can be tolerated and some degree of portability in the apparatus is an advantage.

The arrangement of the reflectance head of the EEL spectrophotometer is shown in Fig. 2. It was slightly modified to hold a standard 1 cm. optical cell blacked on all surfaces but one. The milk level in the optical cell was not critical, but had to be more than 1 cm. above the instrument aperture to prevent extraneous light entering from the surface through the milk.

The EEL spectrophotometer is fitted with a series of nine filters with transmission maxima at various points in the visible spectrum. It is therefore not capable of giving continuous data over a wide range of wave-lengths. It is, however, simple, portable and convenient to use, and will give results generally to a suitable degree of accuracy.

The size of sample required was 10 ml. for the Beckman attachment and 7 ml. for the EEL attachment.

Both instruments require a standard reflecting surface with which the 100% reflectance point at any wave-length can be determined. A block of magnesium carbonate is the accepted standard of reflectance, but it is difficult to maintain in good condition, and a piece of $\frac{3}{16}$ in. white opal glass is more convenient and satisfactory for this type of work. In some experiments it was desirable to measure reflectances relative to unheated milk, and a sample of unheated milk was then used in place of the opal glass.

Method of heat treatment

The samples were contained for heat treatment in thin-walled aluminium tubes 9·5 cm. long and 1·5 cm. diameter with screw tops. The screw tops contained rubber inserts, and the sealing was sufficiently good to permit treatment at temperatures above 130° C. without loss of liquid. An electrically heated oil bath controlled by a contact thermometer was used. 10 ml. samples of water in the aluminium tubes required 150 sec. to reach 119° C. with the oil-bath temperature at 120° C., and 130 sec. to reach 99° C. with the oil-bath temperature at 100° C.

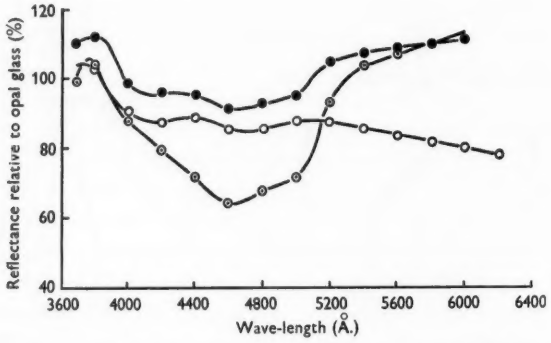

Fig. 3. The spectral variation of reflectance of homogenized milk, separated milk and 40% cream. ●—●, homogenized milk; ○—○, separated milk; ⊙—⊙, 40% cream.

EXPERIMENTAL RESULTS

The effect of heat treatment on the reflectance of milk and cream

The reflectances relative to opal glass of homogenized milk, of the corresponding separated milk and of 40% cream are shown in Fig. 3 for the range of wave-lengths from the near ultra-violet region of the spectrum to the orange-red region. It can be seen how the reflectances of the separated milk and of the cream combine to give the variation in reflectance of the homogenized whole milk.

The effect of heating for various times at 110° C. on the spectral variation of reflectance of homogenized whole milk is shown in Fig. 4. These results were obtained with the Beckman spectrophotometer and are relative to opal glass. The effect at any wave-length is to cause an initial rise in reflectance, followed by an approximately linear fall which is in fact the early part of an exponential decline in reflectance. The fall in reflectance has been shown (1) to correspond to the development of browning as determined subjectively by a panel of observers.

The exponential nature of the fall in reflectance has been noted by Nelson (8). He also noted a reduction in the rate of fall at times shorter than 10 min., which he considered to be a lag in the onset of browning. Since his samples had been evaporated previously, and also placed in a bath at 175° F. for 3 min. for preheating, it is probable that this reduction in rate was the last stages of the initial rise in reflectance. In fact his zero time was after the maximum reflectance had been reached, but before the true exponential browning curve had begun.

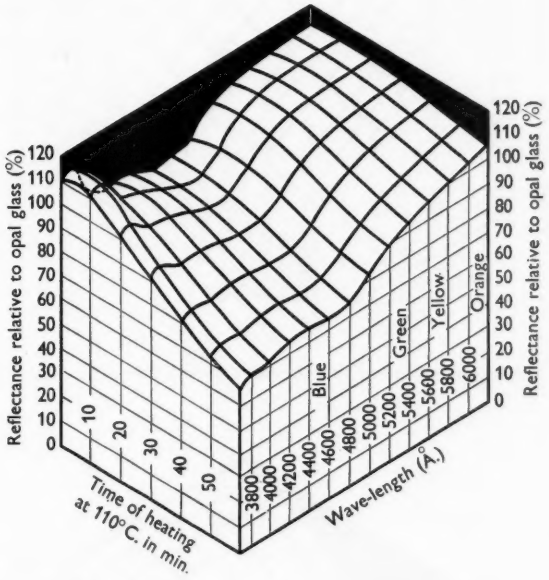

Fig. 4. Variation of reflectance of homogenized whole milk with time of heating at 110° C.

The rise in reflectance occurring in the early stages of heat treatment is a phenomenon entirely separate from that of browning, and will be discussed in a further paper.

The reflectance changes are most pronounced at the ultra-violet and blue end of the spectrum, and are slight and slower to occur at the red end of the spectrum.

Curves corresponding to Fig. 4 for separated milk and for 40% cream heated for various times at 110° C. are given in Figs. 5 and 6. The separated milk shows reflectance changes which are more pronounced than those obtained with homogenized whole milk. The reflectance of the cream is hardly affected, though it still contains 60% of non-fat constituents.

It therefore follows that the colour changes must take place in the non-fat constituents of the milk. The fat phase is little affected by heating, and its presence, even in small proportions, tends to mask the changes taking place in the other constituents.

If it is required only to examine the behaviour of the browning reaction, it is desirable to use separated milk since the sensitivity of measurement is improved. Furthermore, measurements of reflectance taken at the blue end of the spectrum on either whole or separated milk are sufficient to indicate the progress of the colour change, since they are typical of the smaller and slower changes occurring elsewhere in the spectrum.

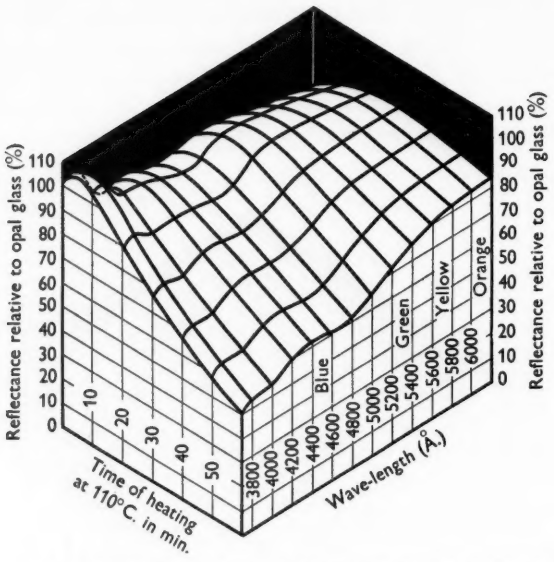

Fig. 5. Variation of reflectance of separated milk with time of heating at 110° C.

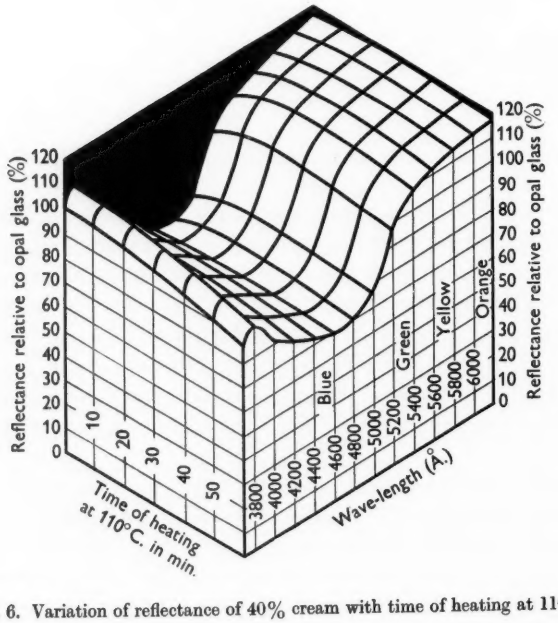

Fig. 6. Variation of reflectance of 40% cream with time of heating at 110° C.

The variation of rate of browning with temperature

The variation of the reflectance of separated milk after treatment at different temperatures for various times is shown in Fig. 7. The reflectances were measured with the EEL spectrophotometer using a no. 601 filter having a peak of transmission in the extreme blue part of the spectrum, at 4260 Å. The results are given relative to the reflectance of the corresponding unheated milk.

The slope of the first part of the browning curve, after the initial rise in reflectance is completed, is approximately linear and is a measure of the rate of browning. The rate can be expressed as a percentage fall in reflectance per unit time.

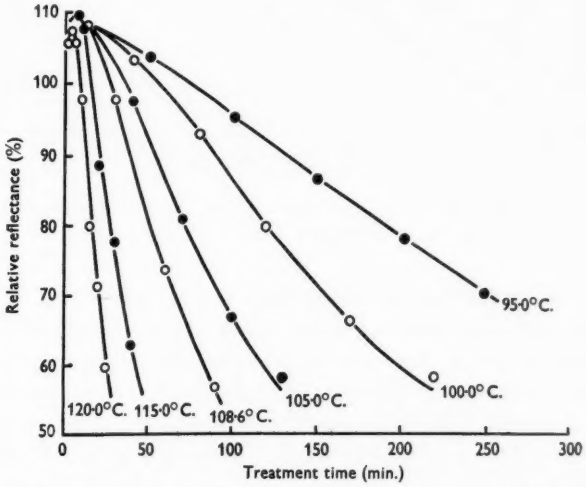

Fig. 7. Variation of reflectance at 4260 Å, with time at different temperatures, for separated milk.

The variation of the logarithm of the rate of browning with temperature is linear, as shown in Fig. 8 (curve *A*). This would be expected over this range of temperatures for a reaction obeying Arrhenius's Law.

Curve *B* of Fig. 8 shows a similar relation obtained by identical methods using homogenized whole milk. Although the magnitude of the reflectance changes differ, the dependence on temperature remains the same, showing that the removal of the fat has not influenced the reaction.

For the comparison of the temperature characteristics of chemical and bacteriological reactions the Q_{10} of the reaction is a convenient criterion. It is defined as the increase in the reaction velocity for a 10° C. rise in temperature, and is almost constant over restricted ranges of temperature well removed from absolute zero. For the temperatures considered here, and probably over the range of temperatures which is of importance in milk treatment, the Q_{10} is constant as shown in Fig. 8. For the separated milk the Q_{10} indicated by the curve is 3·1, and for the homogenized milk it is 2·95.

Change in the wave-length at which the reflectance is measured does not affect the value of Q_{10} obtained within the range 4200 to 5200 Å., while at longer wave-lengths the changes are becoming too small for convenient measurement.

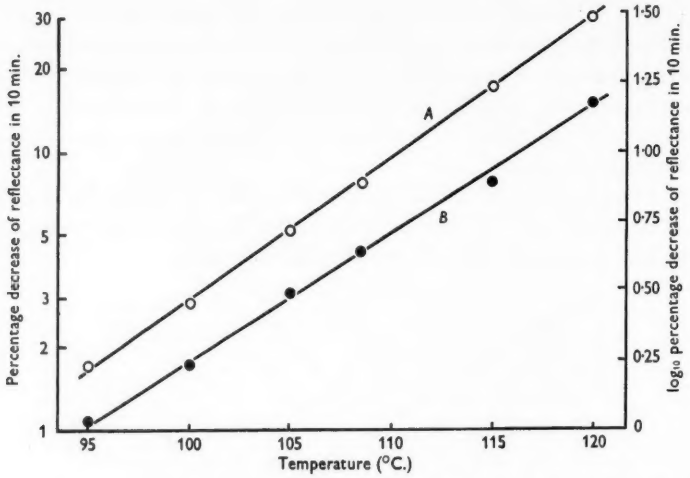

Fig. 8. Variation of rate of browning with temperature. *A*, ○—○, for separated milk from Fig. 7, $Q_{10} = 3{\cdot}1$; *B*, ●—●, for homogenized whole milk, $Q_{10} = 2{\cdot}95$.

The influence of some chemical factors on browning

pH. There has been some doubt as to the way in which pH affects the rate of amino-acid–sugar reactions. Lea & Hannan (9) have shown that the browning of dry casein-glucose mixtures increases with alkalinity, and Patton (10) has shown a similar relation for autoclaved separated milk using the trypsin-digestion method of brownness determination. The reflectance method has therefore been used to give further data on this point.

Fresh separated milk was inoculated with souring organisms, and was incubated to give samples of low pH. Samples of high pH were prepared by the addition of small quantities of N/9 sodium hydroxide.

Samples were heated for various times at 109° C., and their reflectances measured at 4260 Å. relative to unheated milk of the corresponding pH. The resulting variation of relative reflectance with time for a range of pH values is shown in Fig. 9. The variation of rate of browning over the linear part of the curves with pH of the milk is given in Fig. 10.

This curve is similar to that found by Patton, and his curve is also reproduced in Fig. 10. Since there is no determinable ratio between optical densities as found by him and rate of browning as obtained by the reflectance method, his curve has been modified in a constant ratio to give coincidence between the two curves at his point of maximum pH. The similarity between the two curves indicates that both methods show the true influence of pH on the rate of browning of liquid milk.

Formaldehyde. It has been remarked (2) that the addition of formaldehyde inhibits the browning reaction, by providing aldehyde groups for the reaction other than those of the sugars. This can be strikingly demonstrated in separated milk, as shown in Table 1. Although formaldehyde does not prevent browning completely, the addition of quite small amounts reduces the rate of the reaction very considerably.

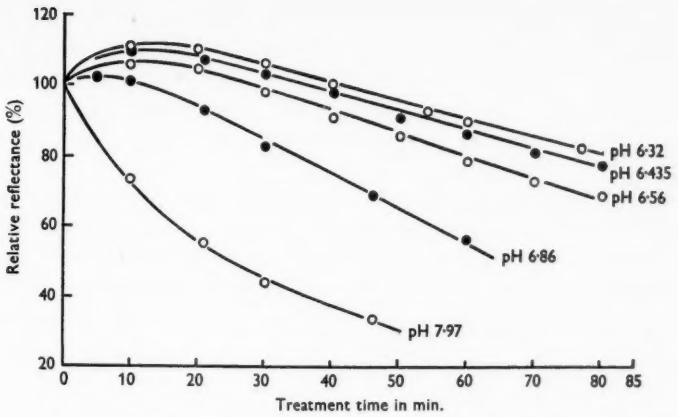

Fig. 9. Variation of reflectance of separated milk at 4260 Å with time at 109° C., for different values of pH.

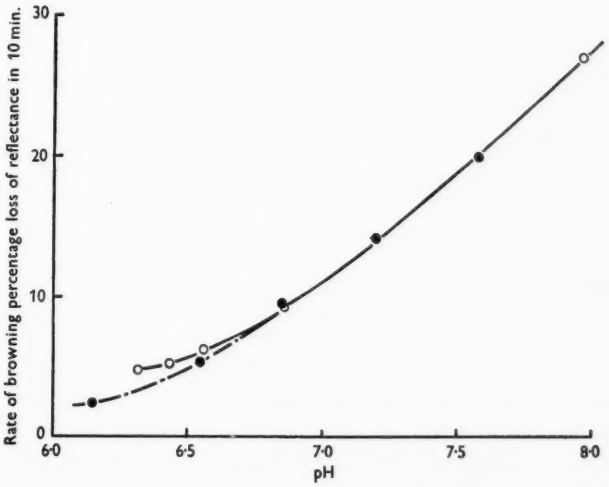

Fig. 10. Variation of rate of browning with pH. ○—○, reflectance measurements; ●—·—●, trypsin digestion (Patton, 1952).

Table 1. *The effect of formaldehyde on the rate of browning of milk*

Formaldehyde (%)	Rate of browning (% decrease of reflectance in 10 min.)
0	3·60
2	0·78
4	0·50
6	0·39
8	0·15

The effect of storage on the colour of sterilized milk

Homogenized milk was heated at 110° C. for approximately 40 min. Samples were stored at 7, 21 and 37° C. and their reflectances relative to opal glass were measured with the Beckman spectrophotometer at intervals. Fig. 11 shows the reflectance of the milk immediately after heat treatment, and the reflectances after 11 days' storage at each temperature.

Except for a slight general drop in reflectance at all wave-lengths, there is no change in the colour of the milk up to 11 days, which is a normal storage period. Storage at the high temperature of 37° C. shows a slightly larger loss of reflectance, but cold storage shows no improvement compared with storage at normal ambient temperatures.

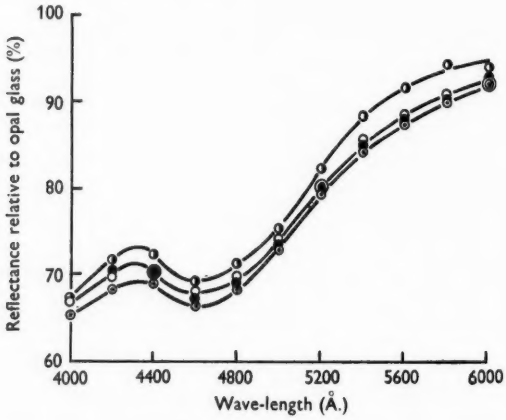

Fig. 11. The effect of storage on the colour of sterilized milk. ◑—◑, measured immediately after treatment; ●—●, after 11 days at 7° C.; ○—○, after 11 days at 21° C.; ⊙—⊙, after 11 days at 37° C.

DISCUSSION

The characteristic of the browning reaction which is of the greatest interest is perhaps its dependence on temperature. The Q_{10} of the reaction is approximately 3, which is comparatively low compared with the figure for resistant spore destruction which is of the order of 10. An increase in milk-treatment temperature with a correspondingly shorter treatment time will therefore give a lower degree of browning for the same bacteriological effectiveness of the process. Higher sterilizing temperatures are therefore an advantage.

A commercial trend towards higher temperatures has already begun, not influenced, as far as is known, by any theoretical considerations.

Higher temperatures cannot be used to full effect with existing in-bottle sterilizing processes. Far-reaching changes in processing techniques will therefore be required to exploit the theoretical advantages.

A further practical consequence of the low Q_{10} for the browning reaction is that significant browning takes place at temperatures which are too low to have any sporicidal effect. In dairies using the batch process of sterilization, where very slow cooling of the milk is common, an appreciable amount of the total browning can occur during the cooling period.

Milk acidity does not restrict, by increasing the susceptibility to browning, the amount of heat treatment that can be used in processing. In fact, the lower the pH the less browning will occur. On the other hand, great care must be taken not to allow milk to become contaminated with alkaline detergents before treatment, since a slight increase in milk alkalinity can cause considerable increase in the rate of browning.

Storage conditions at temperatures below 37° C. have no practical influence on the brown colour after treatment.

Since the colour of milk due to sterilization can be measured very simply with a comparatively inexpensive reflectometer, the method may be found to have applications in routine control of the sterilization process. A simple example of such use is the comparison of the performance of several individual sterilizing autoclaves or continuous sterilizers to detect unsatisfactory operation.

SUMMARY

Methods for measuring the reflectance of milk are described. The effect of heating is to reduce the reflectance of whole and separated milk progressively towards the blue end of the visible spectrum. The reflectance of cream is almost unaffected. The measurement of reflectance at the blue end of the visible spectrum is shown to be a useful method for the determination of the brownness of milk. The variation of rate of browning with temperature and with pH and the effect of storage on brownness are given.

I wish to acknowledge the help given by Mr T. Cheeseman throughout the experimental work recorded here. The figures were prepared for publication by Mr C. Machin.

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612.664.191

551. VARIATIONS IN THE FAT CONTENT OF HUMAN MILK DURING SUCKLING

By W. G. WHITTLESTONE and Mrs DAWN R. PERRIN
Ruakura Animal Research Station, Department of Agriculture, New Zealand

In an earlier paper (1) one of the authors (W. G. W.) reviewed the problem of the change in fat content of cow's milk throughout milking. Attention was drawn to the fact that there is no similar trend in the milk of the sow, and Waller (2) has shown that in the human there is an increase in fat content similar to that which occurs in the cow. The various theories which have been advanced to explain this effect were reviewed and found inadequate. A new explanation was offered in terms of the partial filtration of the clustered fat globules by the ductules. The explanation depends on the globules being clustered under the conditions obtaining within the mammary gland. As this has been a matter for argument (3), it has been thought worth while to examine the association between a rising fat content and creaming at 37° C. for another species. The present study is concerned with the fat globule distribution pattern, fat content and creaming properties of human milk.

EXPERIMENTAL

The creaming of warm human milk. A sample of milk was drawn into a warm thermos bottle and transferred quickly to a creaming tube held at 37° C. in an incubator. After 7 hr. the milk was divided vertically into four equal volumes (8·5 ml.) and the fat content determined. The column was 11·2 cm. high and was divided into four portions at 2·8, 5·6, and 8·4 cm. from the top. Table 1 shows the fat contents of the portions.

Table 1. *The fat content of four different portions of a column of human milk after 7 hr. at 37° C.*

Height of division from top (cm.)	Fat content (%)
2·8	5·06
5·6	3·08
8·4	2·53
11·2	1·10

There is little doubt that creaming occurred at 37° C., and that fat globules are therefore clustered at body temperature.

The fat globule distribution and fat content of serially drawn samples of human milk

Samples of milk drawn at regular intervals throughout suckling were taken at two stages of lactation (65 and 140 days). Table 2 shows the distribution of fat globule sizes and the percentages of fat for these samples. The globule sizes were determined by the camera-lucida method previously described (4). While there was no evidence of a systematic increase in the size of the fat globules, there was an undoubted rise in the fat content of the milk.

Table 2. *Fat globule size distribution and fat contents of samples of human milk taken at regular intervals during suckling at two different stages of lactation*

Sample no.	Fat globule diameters (μ)																Fat contents (%)
	0–0·5	0·5–1·0	1·0–1·5	1·5–2·0	2·0–2·5	2·5–3·0	3·0–3·5	3·5–4·0	4·0–4·5	4·5–5·0	5·0–5·5	5·5–6·0	6·0–6·5	6·5–7·0	7·0–7·5	7·5–8·0	Above 8·0	
	Percentage of fat globules in different size classes																	
Days in milk = 65																		
1	2·5	1·3	9·4	8·2	18·2	12·6	**14·5**	11·9	6·9	3·8	1·9	1·9	0·6	0·6	0·6	—	—	1·21
2	2·1	2·1	2·8	7·0	22·5	**23·2**	14·8	11·3	4·9	4·2	0·7	2·1	1·4	0·7	—	—	—	1·32
3	0·6	1·2	7·0	10·5	11·1	19·3	**20·4**	11·7	4·7	4·7	1·8	2·3	0·6	0·6	1·2	1·2	1·2	1·47
4	0·0	0·7	1·3	8·5	**21·0**	19·1	18·4	15·8	7·9	3·9	2·6	0·7	—	—	—	—	—	2·75
5	0·6	0·6	4·0	5·8	15·6	**19·1**	12·1	15·6	11·6	4·6	4·6	2·3	1·7	0·6	0·6	0·6	—	2·75
6	0·0	0·0	3·2	5·8	19·9	17·3	**21·1**	15·4	10·3	5·8	0·6	0·6	—	—	—	—	—	4·39
Days in milk = 140																		
1	2·2	8·8	9·9	**21·5**	14·3	13·8	13·8	3·9	3·9	2·2	0·6	1·1	1·7	0·6	1·1	—	0·6	1·51
2	0·5	4·3	4·8	6·5	18·3	**19·4**	16·7	11·8	3·8	4·3	3·8	1·1	3·2	0·5	0·5	—	0·5	1·99
3	1·3	5·2	13·0	10·4	15·6	**19·5**	7·1	7·1	5·2	6·5	2·0	2·6	1·3	0·7	2·6	—	—	2·89
4	2·2	10·9	13·6	**18·5**	16·3	11·4	10·3	7·6	5·4	1·6	1·1	—	0·5	—	—	—	0·4	3·48
5	2·1	13·5	9·3	**15·0**	12·9	14·0	7·3	11·4	6·7	2·1	2·6	1·0	1·0	—	0·5	—	0·5	3·81

DISCUSSION

The fact that human milk creams at 37° C. is evidence for the presence of clusters within the mammary gland. The rate of creaming is lower than in cow's milk (1), but it is very much greater than could be attributed to the larger globules migrating as individuals. It is also evident that the rising fat content of human milk cannot be explained by the filtering out of the larger globules. The position of the human glands rules out any gravitational creaming mechanism so that, as in the cow, the theory that the increase in fat content is due to the filtering out of fat globule clusters would seem to fit the facts better than any other theory offered so far.

SUMMARY

It has been shown that human milk, like cow's milk, increases in fat content during suckling. The increase is not associated with any systematic change in fat globule size distribution. Human milk fat globules, like those of the cow, have been shown to cluster at body temperature. These facts support the theory that the increase in fat content during suckling is due to the fine ducts filtering out the larger globule clusters.

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637.127.8

552. HEAT-INDUCED ACIDITY IN MILK

By F. H. GRIMBLEBY

Department of Agricultural Chemistry, The University, Reading

(With 4 Figures)

INTRODUCTION

Increases in the acidity of milk on heating have been observed by several workers who have established the fact that the amount of acid produced is proportional to the temperature and duration of heating. In spite of all the work that has been done on the subject, the main reaction responsible for the production of acid on heating milk and the specific acid or acids formed are not known. Dunscombe (1) showed that on heating milk its titratable acidity increased not only with increasing temperature and time of heating, but also with increasing pH, the presence of alkali in the milk at 80° C. causing a rapid increase in acidity and the development of a brown colour, neither of which were observed in untreated milk at the same temperature. Leeds (2), Whittier & Benton (3,4), Kass & Palmer (5), Gould (6,7) and Gould & Frantz (8) have all shown that as acidity develops in heated milk, increasing amounts of lactose are simultaneously removed, and they consider that lactose is the principal milk constituent implicated in the production of heat-induced acidity.

Gould (6,7) investigated the possibility that the breakdown of lactose to lactic acid and other acids was responsible for the increase in acidity, and although he showed that lactic and formic acids were produced in milk on heating it to 100 and 116° C., the quantities found were far too small to account for the simultaneous loss of lactose. Keeney, Patton & Josephson (11) isolated small quantities of acetic acid from autoclaved skim milk, but in amounts that bore no relation to the amount of lactose destroyed.

Kass & Palmer (5) investigated the possibility that acid production proceeded through the heat-induced combination of lactose and protein, the elimination of basic amino-groups on the protein in this reaction being considered a possible source of the increase in acidity. If basic amino groups were eliminated in this way, their elimination would be reflected in the formol titre and would result in its reduction, but Kass & Palmer found that the formol titre of milk heated at 120° C. was actually greater than that of the unheated milk, and it appeared that the combination of lactose and protein could not account for the observed increase in titratable acidity. Gould & Frantz (8) also found increases in the formol titre of milks heated at 100° C. for periods up to 2·5 hr. and so obtained no direct evidence that a Maillard reaction between lactose and protein was implicated in the production of heat-induced acidity. It should be noted, however, that in these experiments the heat treatment of the milk was so drastic that other heat-induced reactions might be expected to occur which would obscure the effects of lactose and protein combination. In the experiments of Dunscombe (1) the fact that heat-induced acidity increased with increasing pH supports the possibility that a lactose-protein combination was implicated, since this reaction proceeds most rapidly in alkaline

solution when the ionization of the basic amino groups is repressed. Further, Webb & Hufnagel (9) have shown that the addition of reducing sugars to milk increased considerably the amount of acid produced on heating. If, in fact, the combination of lactose and protein is responsible for the production of acidity in heated milk, two stages in the reaction might be expected to exist. Combination of lactose with certain available amino groups in the intact protein, i.e. protein that has not been heated to a temperature at which breakdown occurs, would result in an increase in acidity and a corresponding fall in the formol titre. This would be the first stage. The temperature at which protein breakdown occurs, as evidenced by the liberation of sulphydryl groups is, according to Townley & Gould (10), about 76° C. When therefore the heating is only momentary at temperatures not exceeding about 76° C. there should be an inverse relationship between the titratable acidity and the formol titre. Above this temperature protein breakdown would be responsible for an increase in the number of amino groups available for combination with both lactose and formaldehyde, and both the titratable acidity and formol titre could be expected to increase simultaneously. This would be the second stage. The following experiments were made to obtain more information on these points.

EXPERIMENTAL

Samples of separated raw milk were heated at 60, 70, 80, 90 and 100° C. for periods up to 4 hr. and titratable acidity, pH, formol titre and lactose determined on the heated milks.

Titratable acidity and formol titre were determined on 20 ml. portions by titration with N/10-NaOH to a phenolphthalein end-point matched against 20 ml. milk containing 1 drop of 1 % rosaniline acetate. The usual titration procedure was modified to reduce the effects of fading at the end-point on the formol titre by titrating the milk to a pink end-point, allowing it to stand for 2 min. to allow the fading reaction to proceed, and then completing the titration to an exact match with the colour standard. Without delay, 4 ml. neutral 40 % formalin were added to the neutralized milk and the formol titre carried to a match with the same colour standard. pH was determined by glass electrode.

Lactose was determined by two procedures, colorimetrically using the method of Perry & Doan (12) and polarimetrically on a serum cleared with acid mercuric nitrate.

RESULTS

The results are shown diagrammatically in Figs. 1–4. Those in Figs. 1 and 2 confirm that acidity develops in milk heated at temperatures of 60° C. and above. Within the temperature range 60–80° C. the heat-induced acidity increased rapidly in the first hour and then altered relatively slightly, but at higher temperatures the acidity increased progressively with the duration of heating. The formol titre underwent changes which can be summarized by saying that at all temperatures from 60 to 100° C. there was an initial fall, the duration of the fall being progressively shorter as the temperature was raised. This initial fall was followed by a rise which proceeded at an increasing rate as the temperature was raised. The pH decreased at all temperatures (Fig. 2), the magnitude of the fall increasing with the temperature.

Figs. 3 and 4 suggest that the determination of lactose colorimetrically by the use of sodium picrate appeared to be reliable only in those samples in which thermal decomposition of protein did not take place. At 80° C. and above, the release of sulphydryl groups through the decomposition of protein was probably responsible for the anomalous

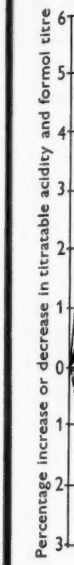

Fig. 1
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Fig. 2
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Fig. 3

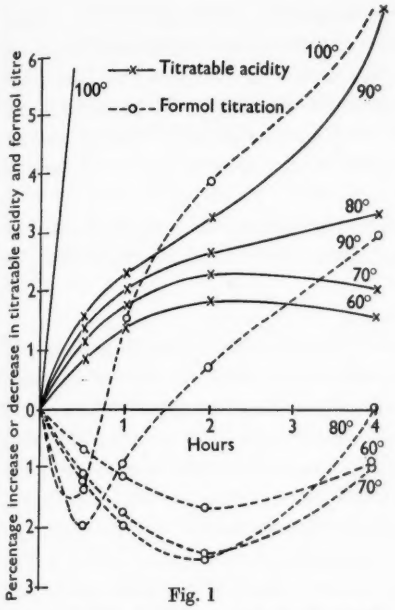

Fig. 1

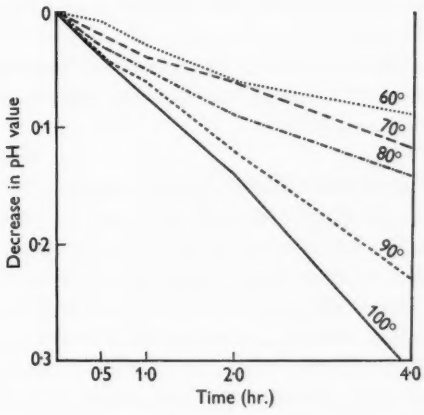

Fig. 2

Fig. 1. Changes in the titratable acidity and formol titre of raw separated milk on heating it at temperatures ranging from 60 to 100° C. for periods up to 4 hr.

Fig. 2. Changes in the pH of raw separated milk on heating it for periods up to 4 hr. at temperatures ranging from 60 to 100° C.

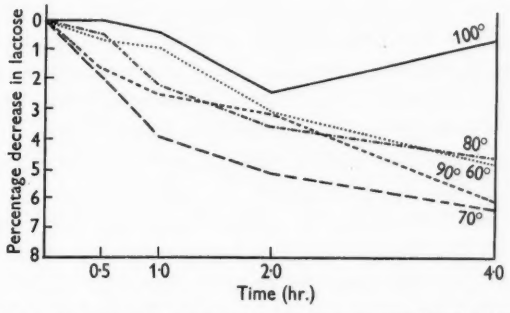

Fig. 3. The effect of heat on the apparent lactose content of raw separated milk determined colorimetrically.

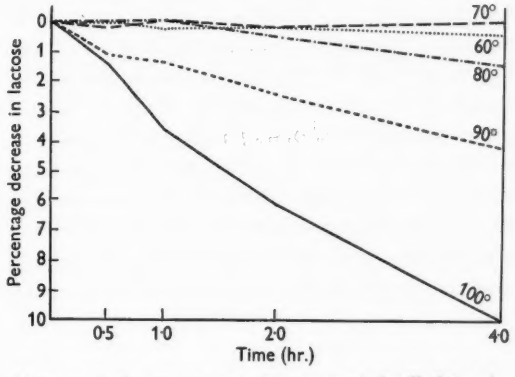

Fig. 4. The effect of heat on the lactose content of raw separated milk determined polarimetrically.

results shown in Fig. 3 for lactose determined colorimetrically. At 60 and 70° C., where protein decomposition was not sufficient to interfere with the colorimetric determination of lactose, the lactose content of the heated samples varied inversely as the titratable acidity. At temperatures of 80, 90 and 100° C., where the colorimetric determination of lactose was inaccurate, the fall in lactose content with increasing heat-induced acidity is best seen from the results obtained by the polarimetric determination of lactose (Fig. 4).

The fact that the lactose values determined polarimetrically in the samples heated at 60 and 70° C. did not show the same variation as those determined colorimetrically is discussed below.

DISCUSSION

The inverse relationship that exists at 60, 70 and 80° C. between the titratable acidity and formol titre of heated milk, and between the titratable acidity and lactose content, indicate that some, if not the major part, of the acidity induced by heat is due to the elimination of basic amino groups attached to protein through their combination with lactose. The combination of lactose and protein produces ultimately humin-like compounds, which are responsible for the brown colour that develops in heated milk (13). At 80° C. and below, there was no browning of the milk, but above 80° C. browning occurred, its intensity being proportional to the temperature and time of heating. The fact that combination of lactose and protein can occur without the simultaneous production of brown colour suggests that the browning reaction proceeds in at least two stages. At temperatures below 80° C. combination of lactose and protein takes place as indicated by the increase in acidity and the simultaneous decreases in formol titre and lactose content. The lactose-protein complexes formed at these lower temperatures are not coloured and are probably decomposed with regeneration of lactose by acid mercuric nitrate, the polarimetric values for lactose showing none of the variations with temperature and time of heating that were shown by the colorimetric values. It would seem that this initial association of lactose and protein is followed by an irreversible chemical binding of the two to form eventually humin-like compounds from which lactose cannot be regenerated by the action of acid mercuric nitrate. Henry, Kon, Lea & White (14) found, during an investigation of the effects of storage on skim milk powder, that the browning of the powder proceeded in two stages, the primary combination of lactose and protein resulting in neither discoloration nor loss of solubility. These two abnormalities followed only as a result of secondary changes.

When milk is heated beyond the point at which thermal decomposition of protein occurs, the development of heat-induced acidity proceeds rapidly. As suggested by Henry, Kon, Lea, Smith & White (15) the combination of lactose and protein probably proceeds through the condensation of the reducing group of lactose with the ϵ-amino groups of lysine and the free terminal α-amino groups of other amino-acids. Also, according to Henry *et al.* (14) the accessibility of such groups is likely to be an important factor in deciding the extent to which combination with lactose takes place. As heat denaturation and thermal decomposition of proteins in milk occur at temperatures above 80° C., not only will acid products be formed but also both the accessibility of available amino groups and their number are likely to increase, and the combination of these amino groups with lactose will be one of the factors permitting the marked increase in acidity noted at these elevated temperatures. The number of amino groups set free at these high

temperatures will probably be in excess of that required to establish an equilibrium with lactose, and an appreciable number of them will be available for combination with formaldehyde and thus give rise to an increase in the formol titre.

It is of interest to note from Figs. 3 and 4 that at 80° C., although some breakdown of protein occurred as shown by the production of reducing substances that interfered with the colorimetric determination of lactose, the heat treatment certainly within the first 1½ hr. was not sufficient to effect the irreversible combination of lactose and protein, so that 80° C. appears to be the highest temperature to which milk can be heated even for very short periods without the production of stable lactose-protein compounds.

SUMMARY

An inverse relationship has been demonstrated between the titratable acidity and formol titre of raw separated milk heated at temperatures of 60, 70 and 80° C. suggesting that the combination of lactose and protein, with the elimination of basic amino groups attached to protein, is one of the main reactions responsible for the heat-induced acidity. At temperatures of 90 and 100° C., when thermal decomposition of protein takes place, heat-induced acidity develops very rapidly and is accompanied by a marked increase in the number of basic amino groups and in the number of these groups which combine with lactose. Below 80° C. the development of acidity was not accompanied by discoloration of the milk, but at 80° C. and above, browning of the milk occurred. The relation between heat-induced acidity and browning is discussed.

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553. MOISTURE LOSSES IN CHEDDAR CHEESE UNDERGOING CURING

By J. K. SCOTT

The Dairy Research Institute, New Zealand

(With 9 Figures)

The effects of a number of conditions on moisture losses of cheese during storage have been known for a long time. A review of existing knowledge is given by Van Slyke & Price (11) who list the following six variables:

(1) Temperature of curing room.
(2) Water vapour in the air of the room (humidity).
(3) Use of paraffin on the cheese.
(4) Size and shape of the cheese.
(5) Moisture content of the cheese.
(6) The quality of the cheese.

The results of experiments reported here give considerably more information about the effects of most of the variables listed. No results are given for waxed cheese, or cheese of variable type or quality, the work in this instance having been confined to the effect of time, moisture, humidity, temperature, size and shape of the cheese in this order.

An increase in moisture content of cheese is known to cause an increase of moisture loss as shown by McDowall (5), who gave the results for 739 Cheddar cheese after 14 days' storage from time of pressing. Very few results have been published about the effect of humidity on moisture losses. A widely quoted experiment is that of Van Slyke who kept one cheese at 60° F. in a room of 75–80 % humidity and one cheese under a bell-jar in which the air was completely saturated with moisture. After 1 month the cheese in the jar had not changed in weight but the cheese in the room had lost 0·76 % of its weight. After 15 months the former cheese had gained 2 % in weight while the room-stored cheese had lost over 11 % in weight. Babcock, Russel & Baer (1) showed that the high humidity of the air which surrounded boxes of stored cheese resulted in a decrease of the rate of moisture loss. Many more investigations of the effect of temperature have been made. Riddet, McDowall & Valentine (6) included moisture-loss measurements in their work on ripening rates, but the results were variable due to humidity variations. Van Slyke (10) compared the losses in weight of cheese stored at 55, 65 and 80° F. Some of the American experiment stations and the U.S. Department of Agriculture investigated the effect of temperature in a general way at the turn of this century. All the results published show that high temperatures cause high moisture losses, but the effects of other variables have prevented the precise effect of temperature being noted. Vernon (13) showed that in New Zealand there is a reduction in moisture loss when cheese is stored in rooms kept at 82 % relative humidity and 55° F. instead of in uncontrolled rooms.

The size and shape of cheese as a variable have been studied by Lane (4) and Van Slyke, Smith & Hart (12) who showed generally that small cheeses lost a greater proportion of their weight than large cheese.

There appears to have been no attempt to show the precise effect of the above variables and to correlate moisture losses generally, and it was with this object in mind that this work was commenced. Most of the results have been confined to the moisture losses which occur during the period of up to 21 days that the cheese is stored at cheese factories in New Zealand. After the initial storage of 14–21 days most cheese made in New Zealand (80 lb. Cheddars) are exported, the full curing taking place during the period between dispatch from the factory and before arrival at the retail market. An account of the conditions during this period is given by Riddet *et al.* (6), but in general terms cheese are stored at about 47° F. for 3 months. Since cheese for waxing are stored for 14 days before waxing, the moisture losses during the first 2 weeks are important.

Relative effects of evaporation and diffusion

The loss of moisture in stored cheese is a special case of drying. Although one normally wishes to reduce the drying rate of the cheese instead of increasing the rate as in conventional drying, the fundamentals of drying theory (8, 9) still apply.

Any particle of water which escapes from the body of the cheese into the surrounding air must first of all travel through the body of the cheese to the surface. From the surface the water particle must diffuse through the air film attached to the cheese surface. The resistance of this film is extremely small compared with the resistance offered by the body of the cheese so that its effects can be neglected, and it is thus possible to consider that the rate of moisture loss is completely controlled by the rate of moisture movement inside the cheese.

The controlling effect of moisture diffusion in the body of the cheese is shown by the change in rate of moisture loss as curing progresses (see Fig. 5) or by the distribution of moisture within a cheese at various times during the curing period. At the start of this investigation a number of cheese were examined for moisture content at various depths below the surface of the cheese. Even after 2 months there was no change in moisture content of samples taken further than 1 in. below the cheese surface. Typical results for an 82 lb. export cheese of initial moisture test of 32·2% and stored for 21 days are as follows:

Distance below barrel surface (in.)	$\frac{1}{4}$	$\frac{3}{4}$	$\frac{5}{8}$	6
Moisture content (%)	25·1	31·2	32·0	32·0

The movement of moisture is therefore so very slow within the cheese that evaporation of the moisture into the air is a comparatively rapid process and, as fast as water can diffuse from the body of the cheese, vaporization is accomplished. As the cheese near the surface becomes depleted of water, diffusion must take place over a longer path, the amount reaching the surface each day becomes progressively less and the rate of moisture loss becomes less with increase of time.

The moisture state

Not all the moisture contained in a cheese is available for evaporation. A portion called bound water is attached to the solid (mainly the proteins) by physical or chemical means and exerts a vapour pressure less than the vapour pressure of free water at the

same temperature. In dairy chemistry there are several definitions of bound water (7), but in this instance the term is defined as above.

The weight of equilibrium moisture held by the solid is less than the amount of bound water for air humidities less than 100%, and the amount usually expressed in lb./lb. of dry solid is termed the equilibrium moisture. Thus if a cheese be placed in an atmosphere of some constant temperature and humidity, the minimum moisture content which continued drying could produce would be the equilibrium moisture. The remaining water, the difference between the total and equilibrium moisture, is the free moisture. The free moisture is thus the water which is available for loss, and the amount depends on the temperature and humidity of the air as well as the total moisture of the cheese.

Equilibrium moistures have been measured for a large number of organic and other materials (3), and in all cases the effect of temperature on the value of equilibrium moisture has been found to be considerably smaller than the effect of humidity. In general, a rise in temperature produces a small decrease in the equilibrium moisture, and with increasing humidity the equilibrium moisture first rises quickly, then increases at a reduced rate, then increases again at a rapid rate. Appendix I deals further with the effect of temperature and humidity on equilibrium moisture.

List of symbols

A	Cross-sectional area normal to direction of flow (ft.^{2}).
a	Constant.
c	Constant.
D	Diameter of cheese (ft.).
e	Epsilon 2·718, etc.
F_0	Free moisture in cheese (lb.).
F/MFS	Percentage fat in moisture-free solids.
H	Height of cheese (ft.).
h	Fractional humidity (dimensionless).
K, K_1, K_2	Constants.
k	Diffusion rate of moisture (ft.^{2}/day).
M	Percentage equilibrium moisture based on dry matter in the cheese.
n	Constant.
R	Absolute temperature (460 + ° F.).
T	Total moisture at any instant (lb.).
T_0	Original total moisture (lb.).
T_s	Total moisture if dried to equilibrium moisture (lb.).
t	Time (days).
v	Absolute viscosity (lb./hr.-ft.).
W	Total loss of moisture at any instant (lb.).
w	Average moisture concentration at any instant (lb./ft.^{3}).
w_0	Initial moisture concentration at any instant (lb./ft.^{3}).
w_s	Surface moisture concentration at any instant (lb./ft.^{3}).
x	Length of drying zone from surface (ft.).

The drying mechanism

The physics of moisture movement in solids (2, 8, 9) has not been precisely determined, and for cheese with its complex physical and chemical character very little is known. In

general, for the drying of solids, two cases are recognized: either (1) the water is not evaporated until it reaches the surface, or (2) the evaporation takes place at a plane below the surface of the solid.

Fig. 1 is a diagram of the general conditions which are believed to exist when drying any hygroscopic solid. Air film effects at the solid surface have not been included. The free water at the surface has a vapour pressure greater than the vapour pressure of the moisture in the air, and the resulting evaporation reduces the moisture concentration of the surface layer until equilibrium moisture is reached. A moisture gradient is then set up between the moisture concentration in the body of the solid and at its surface. If the inflow of heat and the outflow of water vapour are not greatly restricted, the zone of vaporization will recede below the surface and the conditions illustrated are found. Water

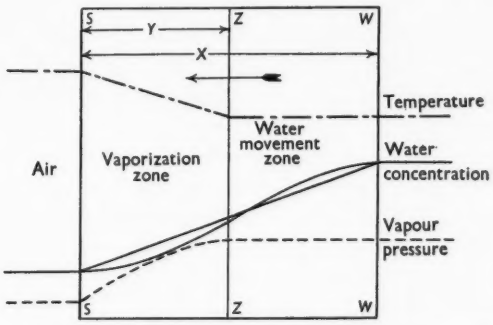

Fig. 1. The general drying conditions for a hygroscopic solid. Water moves under a concentration gradient driving force to a vaporization zone.

moves from plane W–W to plane Z–Z under the influence of a concentration difference. At plane Z–Z water evaporates and the vapour moves to the surface S–S under the influence of the partial pressure driving force. Between the planes W and Z the water concentration values are controlled by the physics of the water movement, but between planes Z and S the water concentration at any point is the equilibrium moisture corresponding to the partial pressure at that point. In some cases the vaporization zone is negligibly small, so that case (1) above can be regarded as a limiting condition of case (2).

It is not known if in the drying of cheese a vaporization zone forms below the cheese surface, but it seems unlikely from the texture of cheese that such a zone exists. This view is supported by general considerations of experimentally determined moisture concentration gradients. Thus for a cheese a simple approach to the analysis of the mechanism is to approximate the moisture gradient to a straight line as shown, and to consider that moisture movement takes place under the action of a linear gradient and an average diffusion rate (8, 14). This procedure should give reasonably accurate results for cheese, since the drying is confined to a relatively thin layer near the surface of the cheese.

It is usual to refer to the movement of moisture through a solid as diffusion even though knowledge of the manner of moisture movement is not clear, and the driving force is taken as the difference in moisture concentrations measured in lb./ft.^{3} of solid volume. Where there is a capillary structure in the solid as in drying a granular solid such as sand, the drying mechanism is entirely different (2), and it is possible for moisture to move from areas of low-moisture to areas of high-moisture concentration.

Appendix II shows that the lb. loss of moisture per lb. of free moisture in a cheese should be related to the time of storage and the size and shape of the cheese by the expression

$$\text{Loss of weight (lb./lb. of free moisture)} = \frac{W}{F_0} = K\left(\frac{kt}{H^2}\right)^c, \qquad (1)$$

where K is a constant for the geometrical shape of cheese, c is a constant at about 0·5, k is the diffusion rate constant in square feet per day, t is the storage time in days, H is some linear dimension, in feet, and taken here as the height of the cheese. It is further shown that for a cheese drying from one face only the value of K is 1·0 and c is 0·5.

Neither air humidity nor air temperature is expressed directly in the above equation, but since humidity affects the equilibrium moisture of the cheese and therefore the free moisture, F_0, changes in humidity give rise to changes in the value of F_0. In a similar way, since temperature has some effect on the equilibrium moisture value, temperature changes also give rise to changed values of F_0. It is known also, from general experience of moisture diffusion in solids, that a rise of temperature of the moist solid will produce an increase in the diffusion rate.

It is difficult to ascertain theoretically the quantitative effect of temperature on the rate of moisture diffusion in cheese because the manner by which the moisture migration takes place is not known. If the flow is through very small pores or orifices, then it seems reasonable that Poiseuille's Law would apply, in which case the rate of moisture flow would be inversely proportional to the viscosity of the aqueous liquid. If moisture movement is by diffusion within the structure of the cheese, it is assumed here that a law similar to the Stokes-Einstein correction for liquid diffusion in a liquid applies, i.e.

$$\text{Diffusion rate is proportional to } \frac{\text{absolute temperature}}{\text{absolute viscosity}}.$$

Over small temperature ranges the effect of the absolute temperature term will be small, and in view of the nature of the assumption can be neglected. Thus, whatever the mode of moisture movement, a probable correlation of moisture loss with temperature can be assumed as

$$\text{Diffusion rate } k \text{ proportional to } \frac{1}{\text{viscosity}},$$

since the temperature affects the viscosity directly.

It is shown later that loss of moisture depends on $k^{0·52}$ and therefore to $(\text{viscosity})^{-0·52}$.

This relation and equation (1) can be used in an attempt to correlate moisture losses in similar bodied cheese of a number of different moisture contents, shapes, sizes, air temperatures and air humidities.

EXPERIMENTAL

For a cheese standing on a shelf, only the barrel surface and the top are in contact with the air, and one would expect that the moisture loss from the flat surface in contact with the wooden shelf would be less than the loss from the other end. In experiments designed to show this difference the barrel surface of a cheese was covered with tinfoil, the cheese being allowed to cure in the normal way, being turned daily. Another cheese from the same vat had one end covered with tinfoil as well as a tinfoil covering of the barrel

surface, the cheese being placed with the tinfoil end resting on the shelf. This latter cheese was not turned at all.

For the first 5–6 days the rate of weight loss of the cheese, which was turned daily, was less than double that of the unturned cheese. Afterwards weight loss of the turned cheese was double that of the other, showing that the equilibrium moisture of cheese in contact with a damp shelf produced a practical difference only for the first 5–6 days. For cheese with the barrel surface exposed the difference is less marked since the large moisture loss from the barrel surface lowers the overall effect. It was concluded that little practical error would result in further experimental work from the fact that one of the cheese surfaces was in contact with a shelf.

After a series of preliminary experiments to test the accuracy of the proposed methods, experiments were made on cheese from a number of vats of curd. The procedure was to make a number of cheese from the curd from a single vat. After removal from the hoops in the morning, the cheese were marked, weighed, and placed in cheese-curing rooms of the experimental factory. Weighings were made on a set of butter scales reserved for this purpose, and the weight each day was noted for a period of 3 weeks. In some cases cheese were weighed at intervals over a longer period. Cheese were turned daily.

Each curing room was fitted with a set of refrigerated finned cooling coils, thermostatically controlled, to give constant air temperature. Air humidity was controlled by a humidistat-operated electric heater. At all times the air temperatures and humidities were measured by recording instruments. No attempt was made to record air velocity which was judged sufficient to maintain even air conditions in the rooms.

For each experiment a sample of cheese was minced and placed in a dish beside the appropriate cheese. The dish with the sample was weighed daily until no further change in weight was observed. The amount of water thus remaining in the cheese was taken to be the equilibrium moisture and was determined by drying the sample in an oven at 101–102° F. for 16 hr.

The conditions for which the rooms were set were:

	A	B	C
Temperature (° F.)	55	55	45
Relative humidity (%)	66	82	82

Cheese of constant height to diameter

The procedure is well illustrated by an experiment begun on 1 September 1952. From a vat three 80 lb. export cheeses (approximately 12·0 in. high by 14·0 in. diameter), three short loaf cheese* (approximately 6·0 in. high by 7·0 in. diameter) and a number of cheese for sampling were made. In each curing room an export, a short loaf and a sampling cheese were placed.

The analysis of the cheese was moisture 35·5%, fat 35% and salt 1·62%. The fat content of the moisture-free solids was 54·2%. Equilibrium moistures from the minced samples were: in the 55°/66% room, 7·9%; in the 55°/82% room, 13·9%; and in the 45°/82% room, 14·1%, based on the percentage of water in the dry solids.

From the initial weight of each cheese and the percentage of moisture in the cheese the weight of total water and dry matter could be apportioned. The weight of equilibrium

* A short loaf cheese is one made in a loaf size hoop, but the cheese is shorter so that it has the same ratio of height to diameter as an export cheese.

moisture could be calculated from the weight of dry matter and by difference the weight of free water in each cheese determined.

Fig. 2 shows the weight lost, expressed in ounces, for the six cheese for a period of 110 days. In Fig. 3 the results are shown plotted as lb. loss of weight per lb. of free moisture for a period of 21 days; humidity as a variable thus being eliminated.

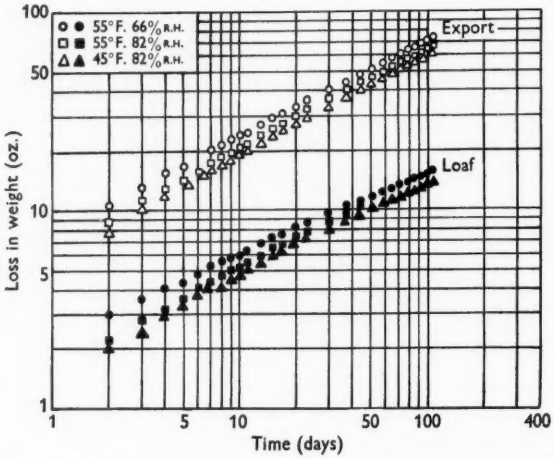

Fig. 2. Loss of weight as a function of time of storage. The linear dimensions of the export cheese are twice those of the loaf cheese.

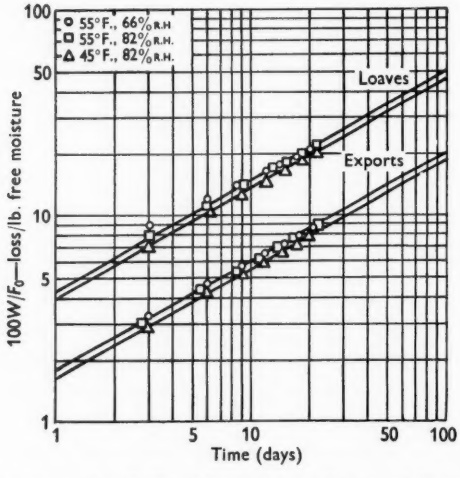

Fig. 3. Loss of weight data expressed as lb./lb. of free moisture, thus correlating the effects of air humidity.

Since the ratio of height to diameter for the short loaf cheese was the same as the ratio for the export cheese it follows from equation (1) that if k the diffusion constant is the same for all cheese, then t/H^2 will be proportional to kt/H^2, and a plot of W/F_0 against t/H^2 should result in single curve irrespective of the size of the cheese. At a lower temperature the diffusion constant will be lower, and the results for this temperature should

plot along a line below that for the higher temperature. The experimental results are plotted in this way in Fig. 4 showing the correlation to hold true, the value of c being 0·535 for this experiment but the average value of c for a number of cheese was 0·52.

The viscosity of water at 45° F. is 1·42 and at 55° F. the viscosity is 1·21 centipoises. From the proposed correlation the moisture loss at 55° F. compared with the loss at 45° F. should be in the ratio of $(1{\cdot}42/1{\cdot}21)^{0{\cdot}52}$ or 1·085. Fig. 4 shows agreement with this value.

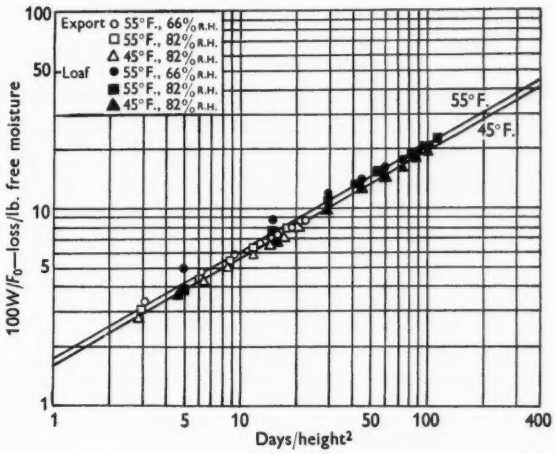

Fig. 4. Loss of weight data plotted against days/height² to correlate the effects of size of cheese.

Table 1. *Values of K_1 in the formula,* $100\ W/F_0 = K_1\ (days)^{0{\cdot}52}$, *for export cheese held at* 55° F.

	Cheese analysis (%)				
Date cheese made	Moisture	Fat	Salt	Fat in moisture-free solids	K_1
4. xii. 51	34·75	36·8	—	56·4	1·58
18. viii. 52	34·50	36·5	1·64	55·8	1·71
1. ix. 52	35·50	35·0	1·62	54·2	1·78
8. ix. 52*	32·98	36·0	1·86	53·8	1·83
29. ix. 52	32·92	36·5	1·85	54·2	1·76
1. ii. 53	35·3	36·0	1·24	55·2	1·80

* This experiment was similar to that commenced on 1 September 1952. The only difference was the lower moisture content of the cheese and the lower fat content. The results were similar to those shown in Figs. 2–4.

Although the correlation confirms the derived expression (1) the particular lines in Fig. 4 hold true only for the diffusion rates in cheese of the body structure appropriate to the vat of cheese tested. Presumably cracking and other body defects would give rise to a faster rate of diffusion and consequently a higher moisture loss. It also seems likely that a cheese with a somewhat smaller value of fat in the moisture-free solids would have a greater rate of diffusion since there would be less fat to impede the path of the moisture. Table 1 shows the results of experiments made mainly during the 1952–3 season.

Examination of Table 1 suggests that there is correlation between the ratio of fat (F) to the moisture-free solids (MFS) and the value of K_1. If the logarithm of the (F/MFS)

ratios be plotted against the logarithm of the values of K_1 the points are scattered about a line having a slope of -2, hence the value of K_1 is inversely proportional to the square of the (F/MFS) over the range considered.

The results to this stage showed that for the normal type of cheddar cheese not suffering from body defects or abnormalities a simple correlation could account for variations in moisture content, fat content, air temperature, air humidity, time of curing and the size of the cheese. Since all tests were made on cheese having approximately the same ratio of height to diameter, only the effect of the shape, i.e. the ratio of height to diameter, needs to be determined in order to derive a general correlation.

Variable height to diameter

In order to show the effect of varying the height to diameter ratio of cheese on the loss of moisture a number of cheese of different shapes and sizes were made on 29 September 1952 from one vat of curd and stored in a room held at 55° F. and 66% humidity. The analysis of the cheese was moisture 32·9%, fat 36·5% and salt 1·85%. The fat content of the moisture-free solids was 54·2%. The equilibrium moisture was 8·15%. Details of the cheese are given in Table 2.

Table 2. *The sizes and weights of the cheese used to determine the effect of shape on moisture loss*

No. of cheese	Size of hoop	Height of cheese (in.)	Ratio: height/ diameter	Weight of cheese (lb.)
50A	Export	12·5	0·87	84·44
50B	Export	9·1	0·62	61·71
50C	Export	6·6	0·47	42·81
50D	Export	3·7	0·26	23·67
50E	Loaf	7·3	1·06	11·07
50F	Loaf	6·6	0·94	9·94
50G	Loaf	5·9	0·87	8·60

Fig. 5 shows the actual loss in weights of the cheese over a period of 21 days, while Fig. 6 shows the loss of weight per lb. of free moisture for each cheese as a function of time/(height)^2. The intercepts K_2 for each line (when $t/H^2=1$) are:

Ratio: $\frac{\text{height}}{\text{diameter}}$	0·26	0·47	0·62	0·87	0·94	1·06
K_2	0·90	1·2	1·42	1·85	2·10	2·30

Values of K_2 can be plotted against values of H/D, so that for any cheese having the same moisture diffusion rate as the cheese tested the value of K_2 can be read from the curve corresponding to the H/D value for the cheese, and can be used to find the loss of weight according to the formula

$$100W/F_0 = K_2(t/H^2)^{0·52}. \tag{2}$$

It can be shown by suitable plotting that the values for K_2 approximate to the expression

$$K_2 = 0·6 + 1·5\,(H/D)^{1·2}. \tag{3}$$

The diffusion rate

Comparison of equations (1) and (2) show that $K_2/100$ in the empirical formula (2) must correspond to $Kk^{0·52}$ in the theoretical formula (1). Thus $K = K_2/(100k^{0·52})$, and if the diffusion rate k is known, values of K_2 can be converted into absolute values of K.

In order to obtain the value of the diffusion constant k pertaining to the values of K_2 given above, two loaf cheese were made, and of each cheese the barrel surface and one end were covered with tinfoil. The cheese were stored, placed on the tinfoil bases, and

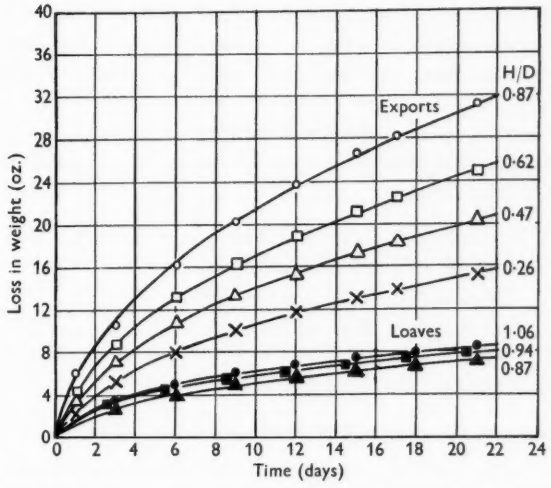

Fig. 5. Loss of weight data for a number of cheese having different ratios of height to diameter and stored at 55° F. and 66% relative humidity.

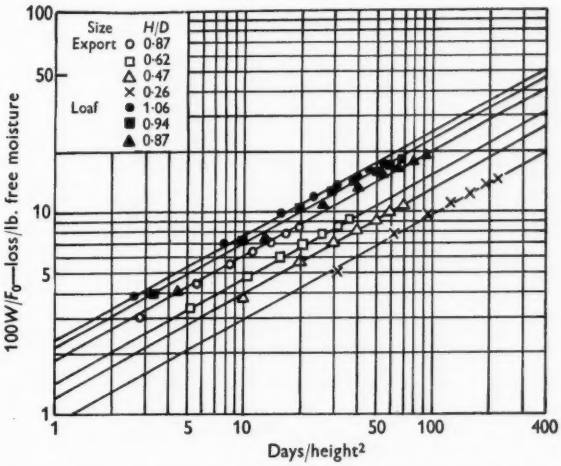

Fig. 6. Data of Fig. 5 replotted to show the effect of shape only on the loss of moisture characteristics.

were not turned at any time, so that moisture evaporation took place at a single plane surface. This case is computed in Appendix II as

$$W/F_0 = 1{\cdot}0\ (kt/H^2)^{0{\cdot}5} = k^{0{\cdot}5}(t^{0{\cdot}5}/H),$$

hence a plot of W/F_0 against $t^{0{\cdot}5}/H$ should result in a straight line from which $k^{0{\cdot}5}$ can be determined. Experimental values of W/F_0 varied as (time)$^{0{\cdot}5}$ for cheese held at 55° F. in

rooms of 66 and 82 % humidity, and a further plot of W/F_0 against $t^{0.5}/H$ gave $k^{0.5} = 0.0045$ and thus $k = 0.00002025$ ft.^{2}/day.

Taking $K = K_2/(100k^{0.52})$ and $100k^{0.52} = 0.368$ we get the following values for K which can be used in the fundamental equation (1):

Ratio: $\frac{\text{height}}{\text{diameter}}$	0·26	0·47	0·62	0·87	0·94	1·06
K	2·44	3·26	3·85	4·48	5·70	6·25

Overall correlation

The foregoing results have shown that for the cheese investigated, the loss of weight depends on the following factors:

F_0	to allow for cheese moisture, air temperature and humidity.
$t^{0.52}$	for the effect of time of storage.
$H^{-1.04}$	to allow for the size of the cheese.
$(F/MFS)^{-2}$	showing the effect of fat content on moisture diffusion rate.
$(1/v)$	for the effect of cheese temperature on moisture diffusion rate.
$(0.6+1.5\,(H/D)^{1.2})$	to allow for the shape of the cheese.

The above factors can be combined to give a resulting relation

$$100\frac{W}{F_0} = 0.33\left[0.6+1.5\left(\frac{H}{D}\right)^{1.2}\right]\left(\frac{F}{MFS}\right)^{-2}\left(\frac{1}{v}\right)^{0.52}\left(\frac{t}{H^2}\right)^{0.52}.$$

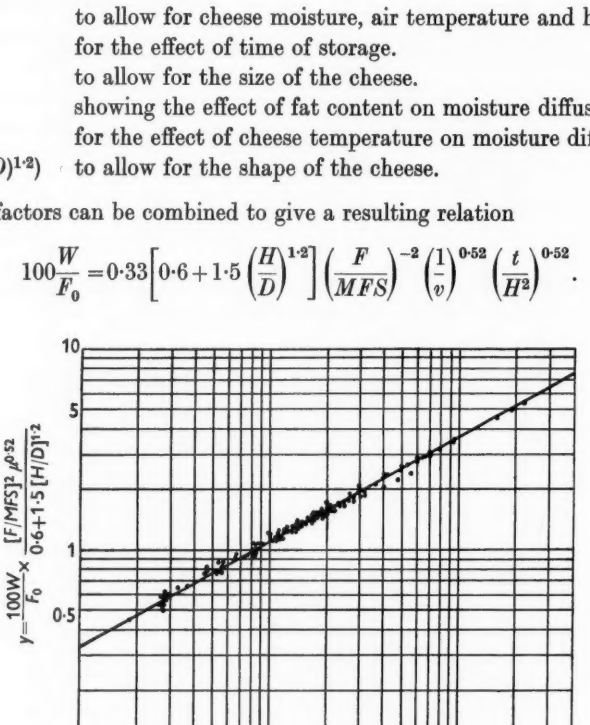

Fig. 7. Correlation of moisture losses for a period of 21 days. The data cover moisture contents of 32·9–35·5 %, fat contents of 53·8–56·4 % in the moisture-free solids; air humidities of 66 and 82 %, air temperatures of 45 and 55° F.; shapes from 0·26 to 1·06 expressed as ratios of height to diameter, and cheese weights of 10–85 lb. The salt contents ranged from 1·62 to 1·86 %.

The correlation of the results of these experiments is shown in Fig. 7.

The plot covers moisture contents of 32·9–35·5 %, fat contents of 53·8–56·4 % in the moisture-free solids, humidities of 66 and 82 %, temperatures of 45 and 55° F., shapes expressed as ratio of height to diameter from 0·26 to 1·06, and cheese weights of 10–85 lb.

DISCUSSION

The results given in Fig. 2 show that cheese held at 66% relative humidity had about 15% more moisture loss than cheese held at 82% humidity. The cheese at 45° F. had about 8% less moisture loss than cheese at 55° F. and the same humidity. It would appear that the change in slope of the lines for the short loaf cheese after 21 days' curing is a function of the extent of the drying of the cheese in relation to its size, the assumption made for the theoretical equation being no longer valid. Had the change been due to a variation in the physical nature of the cheese, then the export cheese would have shown a similar effect. It was found in all the experiments conducted that the moisture loss values within the first 2 or 3 days could be erratic.

The actual value of the slope in Fig. 3 is affected by the initial moisture distribution. When cheese are taken from the hoops the moisture distribution is not uniform, having lower concentrations at the barrel surfaces and the centre of the cheese. It was found that by altering the pressing time a slight change in the value of the slope could be made. Owing to the shrinkage and unequal deformation of the cheese while curing, it was not possible to have the shapes of all the cheese equal, and in view of this the correlation is better than expected.

The size of cheese of most interest in New Zealand is the export size and, assuming an average fat content, the moisture loss in lb./lb. of free moisture can be taken as $1{\cdot}75\ (\text{days})^{0{\cdot}52}$ at a temperature of 55° F. At any other temperature one may use a tentative correction factor based on the value of $(\text{water viscosity})^{0{\cdot}52}$ until the equations presented here have been checked over a wider temperature range:

Temperature (° F.)	40	45	50	55	60	65
Correction factor	0·88	0·92	0·96	1·0	1·04	1·08

To know the actual loss in lb. the weight of free moisture must be known and this can be calculated from the known weight of cheese, moisture content and equilibrium moisture as read from Fig. 8.

A simple calculation, using the equilibrium values given in Fig. 8, will show that the percentage increase in loss due to any low humidity depends only slightly on the moisture content of the cheese, and a table can be prepared taking an average moisture in the cheese of 34%. Thus for a humidity of, say, 80% the equilibrium moisture is 13·0% based on dry matter, and the actual weight of equilibrium moisture is $0{\cdot}130 \times 66$ or 8·45 lb./100 lb., of cheese containing 66 lb. of dry matter. The free moisture is $34 - 8{\cdot}45$ or 25·55 lb., and the free moisture corresponding to any other air humidity can be similarly calculated. Since moisture loss is proportional to free moisture content, the following table giving the ratios of free moistures (taking the value at 82% relative humidity as unity) can be used to estimate the effect of humidity on moisture losses:

Relative humidity (%)	65	70	75	80	82	85	90
Moisture loss factor	1·16	1·13	1·08	1·02	1·0	0·94	0·84

These correction factors are correct for cheese having a moisture content of 34%, and approximately correct for cheese having moisture contents within the usual range.

It is difficult to test the correction factors given in this paper on the results reported in the literature since in most cases the temperature and humidity of the air surrounding the cheese have not been recorded, but where the figures are available the values substantiate the results reported here.

In general, where moisture losses have been reported for cheese cured at different temperatures (but humidities not reported) the increased losses at the higher temperatures are greater than would be indicated by the results of the present experiments, and it is possible that the increase of temperature has been accompanied by a decrease in humidity. For example, cheese held at 60° F. could be expected to have 16% more loss than cheese stored at 40° F. If the reduction in temperature is accompanied by a rise in humidity from 70 to 90% then the cheese cured at the higher temperature (and lower humidity) would have 50% greater loss. It should be noted that cheese stored in boxes in commercial storage can be surrounded by an atmosphere of high humidity, especially if the boxes are stored close together and the air movement is small.

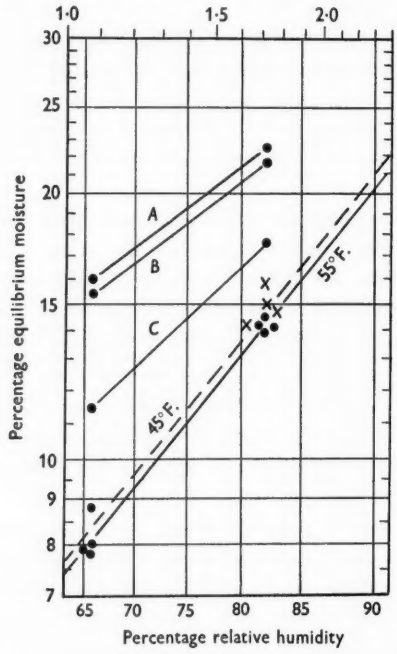

Fig. 8. Values of equilibrium moisture (on dry matter) for fresh cheese at various relative humidities. Full line for the Cheddar cheese held at 55° F., representing average values for the experimental points (full dots). Dotted line for cheese at 45° F. and calculated from 55° F. line. Crosses are experimental points at 45° F. Lines *A*, *B*, *C* are for raw, partly dried and dried lactic casein showing a similar slope to that for the cheese.

SUMMARY

The loss of moisture per pound of free moisture in a cheese is shown to depend on the moisture movement in the cheese and hence on a function of the group (diffusion rate × time/(height)^2). The results of a series of experiments were correlated by means of the above expression. The variables correlated were time of storage, moisture content, fat content, air humidity, air temperature, size and shape of the cheese. Total moisture affects the free moisture value, and air humidity affects the free moisture via the equilibrium moisture. The main effect of temperature is on the rate of moisture diffusion, and

the fat content also affects the rate of moisture diffusion. All experimental results for close-bodied cylindrical cheeses were approximately correlated by the expression

$$\%\frac{\text{moisture loss}}{\text{free moisture}} = 0{\cdot}33\left[0{\cdot}6+1{\cdot}5\left(\frac{H}{D}\right)^{1{\cdot}2}\right]\left(\frac{F}{MFS}\right)^{-2}\left(\frac{1}{\text{visc.}}\right)^{0{\cdot}52}\left(\frac{t}{H^2}\right)^{0{\cdot}52}.$$

A fall in humidity to 66% from 82% increased moisture losses by 15%. A fall in temperature from 55 to 45° F. decreased the moisture losses 8%. Equilibrium moisture was shown to vary only slightly with temperature and at 55° F. values (based on dry matter) were 8% at a relative humidity of 66 and 14% at 82%. A suggested equation is given for equilibrium moistures at other humidities and temperatures. For export cheeses at 55° F. the average moisture loss in lb./lb. of free moisture is given as $1{\cdot}75\ (\text{days})^{0{\cdot}52}$, and suggested correction factors are given for other temperatures, being approximately 8% increase in moisture loss for each 10° F. rise in temperature.

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APPENDIX I

The effect of humidity and temperature on the value of equilibrium moisture

The equations presented in the paper are based on a moisture loss per lb. of free moisture, which in turn depends on values for the total and equilibrium moistures. No intensive study which would allow the direct estimate of equilibrium moisture for a wide range of conditions has been published. For practical use the method suggested by Henderson ((3) in paper) can be used in lieu of more precise information.

Henderson showed that an equation based on Gibbs's thermodynamic relation could represent equilibrium data quite accurately for a wide variety of substances including foodstuffs.

The equation he used was

$$1-h=e^{-aRM^n},$$

where h = relative humidity expressed as a fraction, a, n = constants for each particular substance, R = absolute temperature ° F. + 460, M = percentage equilibrium moisture based on dry matter.

The equation can be arranged as

$$M = \left(\frac{1}{aR}\right)^{1/n} (-\log_e (1-h))^{1/n}.$$

If for some constant temperature R, values of M as found by experiment be plotted against $-\log_e (1-h)$ on log-log paper, then a straight line will result at a slope of $1/n$ and having an intercept on the unit line of $\left(\frac{1}{aR}\right)^{1/n}$. Thus the constants a and n and the effect of temperature can be determined.

Much of the data in the literature does give straight-line plots in this way, even for substances such as spray-dried eggs, wheat, dried prunes and raisins. An investigation requires to be made for cheese, but in the meantime the assumption is made that for any one make of cheese of given fat and protein contents the above relation between equilibrium moisture and humidity holds true.

Fig. 8 shows points plotted in this way, using results from the experiments described in the paper. The full line in the average of values at 55° F., and over the range of humidities of interest gives an equation

$$1-h = e^{-3{\cdot}65} \times 10^{-4} RM^{0{\cdot}833},$$

from which the relation at any temperature can be determined. The dotted line is the line for a temperature of 45° F. calculated in this manner, i.e. $\left(\frac{460+55}{460+45}\right)^{1{\cdot}20} = 1{\cdot}024$, so that the values at 45° F. are 2·4 % greater than those at 55° F. The experimental values of M at 45° F. shown in Fig. 8 do indicate values slightly higher than values at 55° F. That exact correspondence is not obtained between the plotted values, and the dotted line may be due to the difficulty of accurately keeping the two curing rooms at the same humidity. The same figure shows equilibrium values (curves *A*, *B* and *C*) for raw, partly dried and fully dried casein.

In the determination of equilibrium moistures it was found that the values obtained for samples drawn from the centre of a cheese after 4 weeks' curing did not differ significantly from values for samples taken from near the surface of the cheese. Further, it was found that if a cheese were sampled over a period of time, equilibrium values changed, but the extent was not sufficient to alter to any practical degree the amount of free water in the cheese.

In general, equilibrium values tended to decrease from the initial value for a cheese taken from the hoops, with a gradual increase again to a value somewhat higher than the initial equilibrium moisture content. In some cases the minimum was reached after about 6 days, but for some cheese the minimum value was reached 2–3 weeks after commencement of curing. Since it is known that bound water in cheese increases as the time of curing becomes greater, one could expect increasing values of equilibrium moisture and decreasing values of free moisture.

Reference (7) in the paper reports work by Scott Blair, Dix and Wagstaff on vapour pressures of dairy products. Their figures for Cheddar cheese at approximately 60° F. can be compared with the results given in Fig. 8 by converting the vapour pressures into the corresponding air humidities and plotting in a similar fashion. The data for air-dried Cheddar cheese of unspecified salt and fat contents plot along a straight line at a steeper slope than that given in Fig. 8, and the values are somewhat greater than observed in the

present experiments. This is possibly due to the greater maturity of the cheese and the cheese being of lower fat content. The plotted data for the cheese of varying salt contents gave a family of curves of constant slope, but again the equilibrium moisture contents were greater than would have been expected from the results presented here.

The above workers showed that the effect of increasing the salt content of Cheddar cheese from 0·14 to 4·19% is to cause an increase of the equilibrium moistures, and over the small range of salt contents (1·62–1·86%) for the data presented in this paper a similar correlation could be observed between the salt content and the equilibrium moisture for the fat free solids.

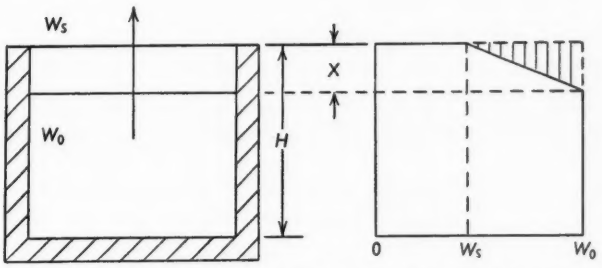

Fig. 9. Diagram of conditions as moisture loss proceeds.

APPENDIX II

Moisture loss by diffusion

Fig. 9 represents a moist solid covered by a moisture-proof membrane on all but one face. The moisture concentration is initially uniform at w_0 lb./ft.^{3}. The solid is placed in contact with air and it is assumed that the moisture content of the surface of the solid rapidly falls to a value w_s equal to the equilibrium moisture for the solid under the temperature and humidity conditions of the air. It is further assumed that the rate of moisture loss from the surface is controlled by the rate at which moisture can move from the interior of the solid to the air-solid interface.

The rate of moisture loss is $\frac{kA(w_0-w_s)}{x}$ lb./day, where k is the diffusivity of moisture in the solid at the particular time in ft.^{2}/day, A is the cross-sectional area of the solid normal to the direction of the moisture movement, in square feet, x is the distance from the surface of the solid to the plane in the solid where evaporation commences, measured in feet.

Hence
$$\frac{dw}{dt} = -\frac{kA(w_0-w_s)}{xAH}, \tag{1}$$

where w = the average concentration of moisture at any instant in lb./ft.^{3}, t = the elapsed time from commencement of drying, in days, H = the length of the solid in the direction of moisture movement, in feet.

Since the area under the moisture distribution diagram represents the weight of moisture in the solid, the shaded area must represent the weight of moisture dried out up to any instant. From the area of this triangle the lb. of water lost are $\frac{(w_0-w_s)\,xA}{2}$ which

must be equal to $w_0HA - wHA$. From this equation

$$x = 2H\frac{w_0 - w}{w_0 - w_s}. \tag{2}$$

Putting equation (2) in (1) for values of x

$$\frac{dw}{dt} = -\frac{k(w_0 - w_s)^2}{2H^2(w_0 - w)} \quad \text{hence} \quad -\frac{(w_0 - w)\,dw}{(w_0 - w_s)^2} = \frac{kdt}{2H^2}.$$

Provided there is no great change in volume of the solid as drying proceeds, the total weights of water can be taken as proportional to the corresponding water concentration, hence using T for total water weights

$$-\frac{(T_0 - T)\,dT}{(T_0 - T_s)^2} = \frac{k\,dt}{2H^2}.$$

The loss in weight (W lb.) is equal to the difference between the initial and instantaneous total weights, or $T_0 - T$ hence $dW = -dT$. The weight of free water F_0 is given by $T_0 - T_s$, hence

$$\int_0^W \frac{W\,dW}{F_0^2} = \int_0^t \frac{k\,dt}{2H^2} \quad \text{and} \quad \frac{W^2}{2F_0^2} = \frac{kt}{2H^2}.$$

Thus

$$\frac{W}{F_0} = \left(\frac{kt}{H^2}\right)^{0.5}. \tag{3}$$

In general, for a solid of any shape the form of the equation can be determined by dimensional analysis. Thus assume the loss of weight can be represented by

$$W = KF_0^a k^b t^c H^d,$$

where K is a constant for the particular shape, H is any linear dimension but here taken as height of the solid.

The basic dimensions are: mass $= m$, length $= l$ and time $= \theta$. $m^1 = Km^a l^{2b} \theta^{-b} \theta^c l^d$, and equating coefficients $a = 1$, $b = c$ and $d = -2c$.

Hence

$$W = KF_0\left(\frac{kt}{H^2}\right)^c. \tag{4}$$

Comparison with the previous analysis shows that for a slab drying from one side $K = 1$ and $c = \frac{1}{2}$ and for a slab drying from two opposite sides the values would be $K = 2$ and $c = \frac{1}{2}$.

Thus the ratio of the change in weight to the possible change in weight is a function of the dimensionless group $\left(\frac{kt}{H^2}\right)$, just as in heat transfer analysis the ratio of temperature change is some function of the same dimensionless group (the Fourier Number) in which diffusivity is replaced by thermal diffusivity.

The classical treatment of the diffusion equations is available in a number of textbooks and papers, and results in a series involving the above dimensionless group. Owing to the small amount of drying which takes place in cheese curing it was considered better to derive the simpler equations shown above.

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554. TITRATION CURVES OF WHEY CONSTITUENTS*

By M. BOULET and DYSON ROSE

Division of Applied Biology, National Research Council, Ottawa, Canada

(With 5 Figures)

INTRODUCTION

Many of the least understood properties of milk, including the precipitation of milk solids during frozen storage, appear to be related to soluble calcium and to the complexities of the calcium phosphate-caseinate system. Fundamental studies of the calcium compounds were therefore undertaken as part of an investigation of the storage of frozen milk and milk concentrates.

Van Slyke & Bosworth observed (19) that addition of potassium oxalate reduced the amount of base required to bring the pH of milk to the phenolphthalein end-point. This effect was re-interpreted by Pyne & Ryan (14, 15), and was adapted by Ling (12) in a method for the determination of colloidal calcium phosphate. Ling's method has been criticized by ter Horst (8), but Pyne & Ryan (16) consider that with minor modifications the method gives a reasonably accurate value. However, no thorough studies of the reaction involved appear to have been made. Whittier (22) and Wiley (23) have investigated the buffer action of some milk constituents, and Eilers (1) has studied the precipitation of di- and tricalcium phosphate. Both Wiley (23) and Eilers (1) gave titration curves for milk and whey constituents, but only in the absence of oxalate.

The amount of base required for the titration of soluble phosphate from pH 6·6 to 9·5 or 10·0 depends upon the amount of calcium present. In the absence of calcium, only a trace of the tertiary hydrogen is titrated, and the base consumed is equivalent to the secondary hydrogen of the monobasic phosphate originally present. In the presence of an isoionic concentration of calcium, precipitation of phosphate begins at about pH 5·0, and is complete at pH 6·0 (5, 7). Since the insoluble salt formed is normally tricalcium phosphate, the base consumed is equivalent to the secondary hydrogen of the original monobasic phosphate plus the tertiary hydrogen of all of the phosphate precipitated. The difference between the amounts of alkali required to reach a given pH value, in the absence and presence of calcium, is thus a measure of the tricalcium phosphate precipitated. When the difference between the titrations becomes constant, precipitation of calcium is complete. However, dicalcium phosphate can sometimes be precipitated (7, 21) and precipitation of this salt does not require extra base over that used in the absence of calcium.

In the presence of limiting amounts of calcium, precipitation begins when either (7)

$$K_2 = [Ca^{2+}]\,[HPO_4^{2-}] = 1 \times 10^{-6}$$

$$\text{or } K_3 = [Ca^{2+}]^3\,[PO_4^{3-}]^2 = 1 \times 10^{-26}$$

is exceeded, but may be temporarily delayed by formation of supersaturated solutions.

* Contribution from the Division of Applied Biology, National Research Council, Ottawa, Canada. Issued as paper No. 298 of the Canadian Committee on Food Preservation, and as N.R.C. No. 3289.

At a fixed total phosphate level the concentration of HPO_4^{2-} and PO_4^{3-} is a function of pH, and precipitation in the presence of limiting calcium will therefore occur above a definite 'saturation' pH. The presence of an anion such as citrate (3), capable of forming a weakly ionized calcium salt, reduces the effective calcium ion concentration and increases the concentration of HPO_4^{2-} or PO_4^{3-} required to exceed the solubility products. Such anions thus raise the 'saturation' pH, or as expressed by Wiley (23), shift the 'calcium buffer effect' towards the alkaline side.

In rennet whey from normal milk, calcium is present in less than isoionic concentration, while in oxalated whey calcium is absent. Assuming precipitation of tricalcium phosphate during titration, titration curves for whey and oxalated whey will begin to diverge at the saturation pH, and the difference will increase with increasing pH until precipitation of tricalcium phosphate ceases. The curves will then run parallel until approximately pH 10·0 is reached, but because the oxalated whey now contains more phosphate, the two curves will converge again as the tertiary phosphate is titrated at still higher pH levels.

The present paper shows the effect of varying phosphate, citrate and whey protein concentration on the titration curves and saturation pH of artificial and natural milk sera. The results are discussed in terms of the solubility products of calcium phosphates in milk, and in terms of the titrimetric estimation of colloidal phosphate (12).

MATERIALS AND PROCEDURE

Solutions of the major salts of milk serum were prepared from reagent grade chemicals. Most of the solutions were variants of an artificial serum containing 8·1 mM. of calcium, 8·1 mM. of phosphate, and 10·2 mM. of citrate per litre, but sodium sulphate was added to some solutions to provide a constant ion concentration. Twenty-five millilitre aliquots of these artificial sera and of rennet whey prepared by Ling's (12) procedure were titrated from pH 6·5 to 10·5 in the presence and absence of oxalate. The difference between the two curves is reported as 'millilitres of excess base'.

The titration assembly consisted of a Beckman Model G pH meter with a general purpose glass electrode, a magnetic stirrer, and a 5 ml. microburette containing 0·2 N sodium hydroxide. The system was standardized with buffer (pH 10·0) before and after each titration, and if drifting occurred the results for that titration were discarded. Errors caused by deposition of insoluble salts on the electrode surface were minimized by rinsing the electrode between titrations in water, 0·1 N hydrochloric acid, water, 0·1 N sodium hydroxide, and finally in several changes of water. After each addition of alkali, the pH was read at 5 min. intervals until no further change was detected. This equilibration period varied from 10 to 45 min.

EXPERIMENTAL AND RESULTS

Effect of phosphate

A series of artificial sera having constant calcium (8·1 mM./l.) and citrate (10·2 mM./l.) contents but with phosphate contents of 3·24, 4·84, 6·48, 8·10, 9·72 and 13·00 mM./l. were titrated in the presence and absence of oxalate (Fig. 1). Smooth curves of relatively uniform shape were obtained, and the total consumption of base increased progressively with increasing phosphate as expected.

The curves indicating the amount of 'excess base' used to precipitate a calcium phosphate varied markedly with phosphate concentration. Solutions containing from 3·24 to

8·10 mM./l. of phosphate gave 'excess base' curves which showed a slight change in slope above pH 9·0, but the consumption of excess base in the presence of calcium continued to pH 10·5. This continued consumption of excess base indicated that tricalcium phosphate was being precipitated throughout the titration range. The highest consumption of excess base (0·52 ml. in the solution containing 8·1 mM./l.) was equivalent to only 6·2 mM. of calcium per litre or 77 % of the total.

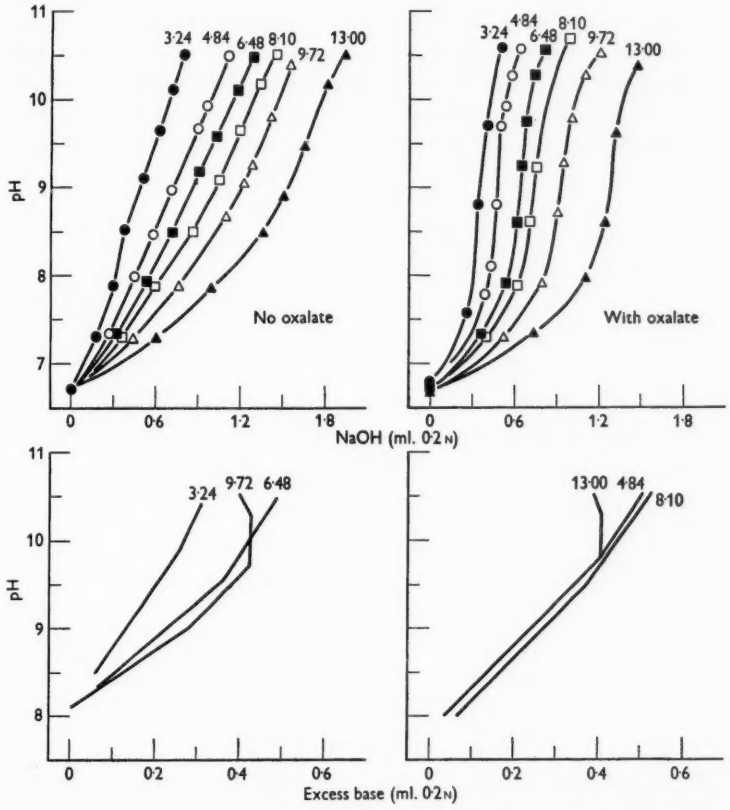

Fig. 1. Titration curves in the presence and absence of calcium for solutions of varying phosphate content. (Numbers on curves give phosphate content in mM./l.)

The 'excess base' curves for solutions containing 9·72 and 13·00 mM./l. phosphate were similar to those at lower phosphate levels up to pH 9·7, but showed a distinct 'break' at this pH. Between pH 9·7 and 10·3 (where titration of the remaining tertiary phosphate apparently began) these curves were parallel to the ordinate, indicating that precipitation of tricalcium phosphate had ceased. In these solutions phosphate was present in considerable excess of the amount required to precipitate all of the calcium as tricalcium phosphate, but nevertheless the excess base consumption (0·42 and 0·41 ml.) was much less than the theoretical amount (0·675 ml.) for precipitation of the calcium. Since a constant 'excess base' value indicates that all of the calcium has been precipitated, use of less than the theoretical amount of base indicates some precipitation of dicalcium

phosphate. A mixed precipitate must therefore have been present, but equilibrium between precipitated salts is established so slowly (21) that disequilibria would not have been detected in the 5 min. periods allowed between pH measurements.

Effect of citrate

The effect of varied citrate content is shown in Figs. 2 and 3. The solutions used to obtain the data of Fig. 2 had a calcium and phosphate content of 8·1 mM./l., and citrate alone was varied. Calcium-free solutions were obtained by the addition of oxalate. In the solutions of Fig. 3, sulphate was added to replace citrate, and the use of oxalate was avoided by preparing the solutions from acids and adding calcium, as the hydroxide, to only a portion of each solution. The total amount of base used (calcium plus sodium hydroxide) was recorded, but the curves are shown only from pH 6·0 upwards.

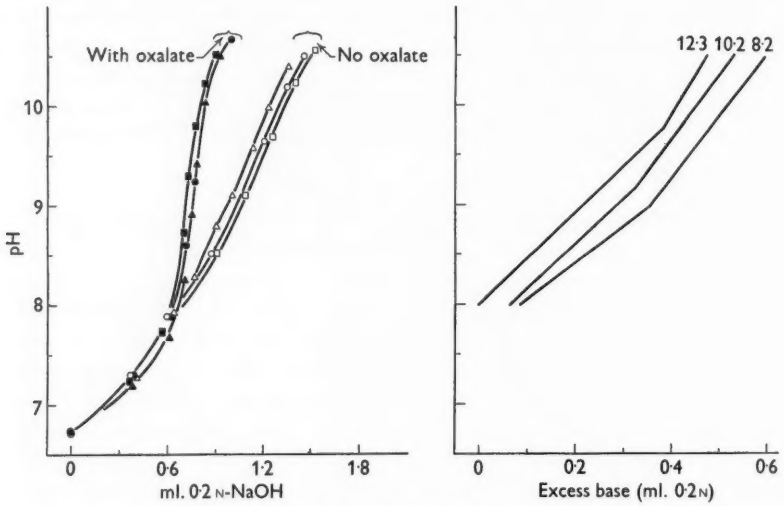

Fig. 2. Titration curves for artificial sera of varied citrate content.
(□, ■, 8·2; ○, ●, 10·2; △, ▲, 12·3 mM./l. citrate.)

Although citric acid is entirely neutralized below pH 7·0, increasing citrate markedly decreased the consumption of excess base (Fig. 2). None of the three 'excess base' curves showed an end-point for calcium precipitation, indicating that calcium was retained in solution up to pH 10·5. The base consumption to pH 10·0 corresponded to 4·9, 5·4 and 6·1 mM. of calcium per litre at citrate levels of 12·3, 10·2 and 8·2 respectively; thus more calcium was retained in solution at the higher citrate levels.

Maintenance of a constant ion concentration and the use of a wider range of citrate concentrations gave stronger evidence of the effect of citrate on calcium solubility (Fig. 3). In the absence of citrate, the saturation pH, i.e. the pH at which the solubility product of calcium phosphate was exceeded, was approximately 6·0, but a supersaturated solution was maintained to pH 6·7. A citrate concentration of 6·5 mM./l. raised the saturation pH to 7·5, and 13·1 mM./l. raised it to 8·6. In the absence of citrate, precipitation was complete at pH 6·7 (theoretical base required 0·675 ml., base used 0·650 ml.), but in its presence precipitation was not complete at pH 10·0. The base consumption at pH 10·0 in

the presence of 6·5 and 13·1 mM./l. citrate was 0·52 and 0·35 ml. respectively, corresponding to 77 and 52% of the calcium present.

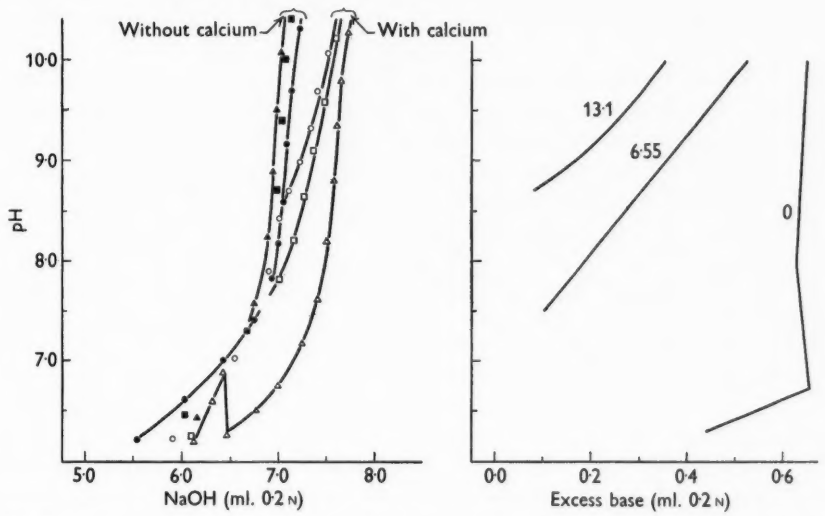

Fig. 3. Titration curves for artificial sera of constant ion concentration but varied citrate content. (○, ●, 13·1; □, ■, 6·55; △, ▲, 0 mM./l. citrate.)

Effect of whey protein

Curves showing the excess base consumption for artificial sera containing whey proteins (prepared according to Harland & Ashworth (2), then lyophilized), are shown in Fig. 4. Increasing concentrations of protein progressively increased the 'excess base' value throughout the pH range 7·5–10·0. At the lower protein levels no end-point for precipitation was reached, but in the presence of 0·8% whey protein, precipitation appeared to be complete at pH 10·0. However, the consumption of excess base corresponded to only 77% of the calcium present, indicating that some dicalcium phosphate had been precipitated. Thus, although whey protein reduced the effect of citrate, complete precipitation of calcium was not attained at the lower protein levels, and even at the highest level calcium was not quantitatively precipitated as tricalcium phosphate.

Titration of rennet wheys

Because rennet whey contains citrate at approximately the concentration used in the artificial sera, the addition of more citrate would be expected to have little effect on the titration. The effect of a second calcium sequestering agent, tetrasodium pyrophosphate, was therefore studied (Fig. 5A). In the absence of pyrophosphate the titration curves for rennet whey were similar to those obtained with some of the artificial sera. The excess base consumption, which was complete at pH 9·5, corresponded to 9·0 mM. calcium per litre. Addition of sequestering agent raised the saturation pH from 8·0 to 9·2, and delayed precipitation to the extent that no end-point was reached.

Titration curves of rennet whey from pasteurized skim milk are given in Fig. 5B. These curves show that the phenomena encountered in artificial sera also occur in rennet

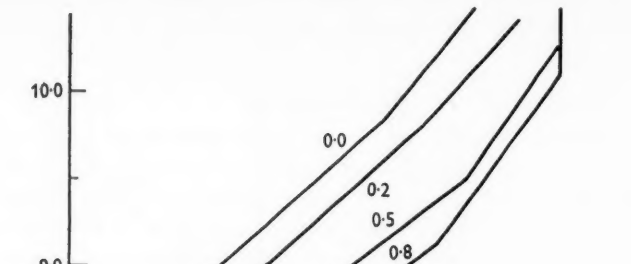

Fig. 4. Curves showing the excess base consumption in artificial sera containing whey protein. (Numbers on curves give the whey protein content in per cent.)

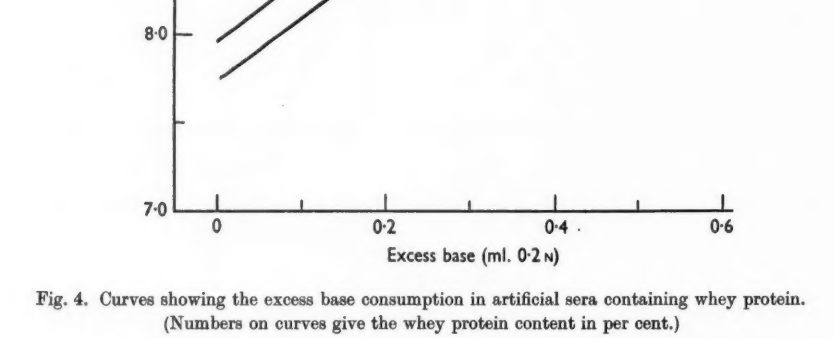

Fig. 5. Curves showing the excess base consumption in rennet whey (A) with added pyrophosphate, and (B) from fresh and stored (3 days, 40° F.) milk.

wheys from normal milk. Thus, in whey from fresh milk, precipitation of calcium continued up to pH 10·0, and no end-point for the consumption of excess base was reached while in the whey from stored milk (3 days at 40° F.) an end-point was reached at pH 9·5.

DISCUSSION

The soluble calcium content of milk, which has been shown by numerous analyses (11, 13, 20) to be approximately 300–400 p.p.m., greatly exceeds the amount of calcium at saturation as calculated from the reported solubility product of di- or tricalcium phosphate and the dissociation constant of calcium citrate. Assuming a saturation pH of 6·5, a soluble inorganic phosphate content of 8·1 mM./l., dissociation constants for phosphoric acid of $1{\cdot}15 \times 10^{-2}$, $1{\cdot}68 \times 10^{-7}$ and $2{\cdot}34 \times 10^{-12}$ (4), and a solubility product for tricalcium phosphate of 1×10^{-26} (7), the free calcium ion content would be $2{\cdot}8 \times 10^{-4}$ M./l. Substituting this value in the equation for the dissociation of $CaCit^-$, and assuming 10·2 mM./l. total citrate, and a dissociation constant of the $CaCit^-$ ion of $6{\cdot}3 \times 10^{-4}$ (3), the concentration of $CaCit^-$ ion is $3{\cdot}17 \times 10^{-3}$ M./l. Total soluble calcium (Ca^{2+} plus $CaCit^-$) by calculation would thus be approximately 140 p.p.m. Similar calculations based on a saturation pH of 7·0 gives a soluble calcium value of only 54 p.p.m.

Seekles & Smeets (18), using ammonium purpurate (murexide) as a calcium indicator, obtained an average value of $2{\cdot}75 \times 10^{-3}$ M./l. calcium ion in milk ultrafiltrate. Substituting this value in the equation for the dissociation of $CaCit^-$, as above, gives a $CaCit^-$ concentration of $8{\cdot}5 \times 10^{-3}$ M./l., and thus a total soluble calcium of 450 p.p.m. Since the low dissociation of calcium murexide decreases the calcium ion concentration and thereby increases the dissociation of $CaCit^-$, a high value for calcium ion is inherent in the murexide method. Nevertheless, the calculated total soluble calcium based on Seekles & Smeets's figure is much closer to the actual value than that based on the solubility product of tricalcium phosphate. This suggests that the solubility product for tricalcium phosphate, and not the dissociation constant for $CaCit^-$, is incorrect under the conditions existing in milk.

Various other whey constituents might contribute to the solubility of calcium, but their low concentrations make a major contribution unlikely. For example, serum albumin binds calcium, but estimates based on recent data (9) indicate that at a calcium ion concentration of $2{\cdot}8 \times 10^{-4}$ considerably less than 10 p.p.m. calcium could be bound by the concentration of lactalbumin present in whey. Moreover, the data of Fig. 4 indicate that in the presence of citrate calcium solubility is slightly depressed by lyophilized lactalbumin.

Additional evidence that other whey constituents have little influence on calcium solubility is supplied by the work with artificial sera. Solutions of 8·1 mM./l. phosphate and calcium (250 p.p.m. P, 325 p.p.m. Ca) are easily prepared in the presence of 10·2 mM./l. citrate, and show no evidence of being either supersaturated solutions or colloidal suspensions. Solutions containing 400 p.p.m. P and 325 p.p.m. Ca have also been prepared, and no release of base was observed upon the addition of oxalate (Fig. 1), indicating that no calcium-phosphate precipitate was present. It must therefore be concluded that the solubility of calcium in citrate-phosphate mixtures is much greater than that indicated by the dissociation constants and solubility products (cf. also (17)). This increased calcium solubility would result if the solubility of phosphate salts were higher, or the dissociation constant for calcium citrate lower, than the accepted values, or if an interaction occurred

between phosphate and citrate so as to reduce effectively the level of phosphate ion. Although phosphate promotes the precipitation of calcium citrate (10), a significant binding of phosphate by citrate in solution appears improbable. Binding of citrate by lactalbumin is somewhat less improbable, and might explain the anomalous effect of added lactalbumin (Fig. 4). A detailed study of the physical constants applicable to these mixtures has been undertaken.

The data presented in this paper also contribute to an understanding of the reactions involved in the Ling (12) titration. The Ling method involves four titrations to a pH slightly below 10·0 (6). The values obtained (M_1 for milk, W_1 for whey, M_2 for oxalated milk, and W_2 for oxalated whey) are interpreted as follows:

$$M_1 = C + S + O, \quad M_2 = C + S - X,$$
$$W_1 = S + O, \quad W_2 = S,$$

where C = curd acidity, S = serum acidity, O = 'overrun', i.e. the excess base required to precipitate tricalcium phosphate, and X = the base released by colloidal phosphate in the following reactions:

$$Ca\,(PO_4)_2 + 3K_2\,\text{oxalate} \rightarrow 3Ca\,\text{oxalate} + 2K_3PO_4,$$
$$K_3PO_4 + H_2O \rightarrow K_2HPO_4 + KOH.$$

For the 'O' value to be meaningful, precipitation of calcium phosphate must occur only in the tricalcium form, and must be complete at or below pH 10·0. The data presented indicate that neither of these requirements is satisfied in some sera, and that precipitation is not always complete in natural whey.

For the 'X' value to be accurate the 'O' included in M_1 and W_1 must be identical. Eiler's data (1) indicate that casein promotes formation of tricalcium phosphate in preference to dicalcium, and there is thus more likelihood of dicalcium phosphate forming in whey than in milk. A lower 'O' value in W_1 than in M_1 would make 'X' erroneously high. This error is, of course, independent of the modifications in the method suggested by Pyne & Ryan (16).

Since it requires several hours to obtain data for titration curves of the type presented in this paper, it is impractical to study many milk or whey samples. However, the difference between fresh and stored whey (Fig. 5) has been observed with several samples, and is apparently of real significance. This difference could be caused by either a decreased soluble calcium or an increased precipitation of di- in preference to tricalcium phosphate, but it cannot at present be fully interpreted in terms of the chemical equilibria in milk.

SUMMARY

Titration curves of calcium-containing and calcium-free solutions resembling milk serum indicated that precipitation of calcium from such solutions was greatly impeded by citrate. In the absence of citrate, precipitation of tricalcium phosphate was complete at pH 6·0, but, in solutions containing citrate, precipitation of tricalcium phosphate occurred gradually throughout the titration and was not complete at pH 10.

In some solutions precipitation of calcium phosphate ceased at about pH 9·7, even though the base added had been insufficient to neutralize tertiary hydrogen equivalent to the known calcium content. Precipitation of dicalcium phosphate must therefore have occurred.

The observed stability of calcium in these solutions was much greater than that predicted from the accepted solubility and dissociation constants. It is therefore concluded that detailed studies of these constants, and of the factors controlling precipitation of di- or tricalcium phosphate, are needed.

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555. AROMA-PRODUCING GROUP 'N' STREPTOCOCCI ISOLATED FROM CHEDDAR CHEESE CURD

By M. ZIELINSKA and E. R. HISCOX

National Institute for Research in Dairying, University of Reading

(With 1 Figure)

It is now well known that acetic acid, CO_2 and C_4-compounds in milk are produced by some streptococci as well as by *Leuconostoc* spp. Several authors (1-5), having isolated them, and measured the amounts of acetoin and diacetyl produced, advocated their inclusion in starter cultures.

Swartling placed them (5) in the serological group 'N' and suggested a relationship to *Streptococcus lactis*.

The work reported here is part of a study of organisms isolated from Cheddar cheese (6). The streptococci described were isolated from cheese curd before salting. They dominated the flora of the curd and were no doubt introduced with the starter. Although they were believed to be *Str. lactis* or *Str. cremoris* their identity was not further investigated at that time. It had been noted that the starter used in the manufacture of these cheese had shown slight gassiness (not due to coliform organisms), and frequently small gas bubbles were found in the coagulated milk cultures of the freshly isolated streptococci. Later tests showed that, unlike typical strains of *Str. lactis*, most of those strains isolated from the cheese curd produced appreciable amounts of volatile acids, CO_2 and acetoin-diacetyl in milk.

MATERIAL AND METHODS

Forty-eight strains of 'N' streptococci from cheese curd (C_{30}) (6) were studied: also twenty strains of *Str. lactis* and *Str. cremoris* from the N.I.R.D. collection, one strain of *Str. citrophilus* P.R.M./1 from Dr Pette and one strain of *Str. diacetilactis* (Matuszewski) received from Dr Pijanowski.

Nineteen strains of group 'N' streptococci were also isolated from a Danish starter culture kept at the N.I.R.D. and tested for production of acetoin.

Acetoin and diacetyl production

Barritt's modification of the V.P. test (7), being more sensitive, was used instead of the usual creatine method (8), but in some cases the latter was also used for comparison. The cultures were grown in the inclined position at 30° C. in citrate-lactose and lactose broth (5), in raw, pasteurized and sterilized milk and cream, and tested for acetoin after 24 hr., 48 hr. and 14 days.

Quantitative determinations were made using der Herder's (9) modification of the method of Prill & Hammer (10). Acetoin was oxidized to diacetyl with 40% ferric chloride. The intensity of colour developed in the Barritt test was found to give groupings of the strains which agreed well with those obtained by der Herder's method. The Barritt test was therefore substituted for the long quantitative method for comparisons of several cultures at one time.

Volatile acid production

Volatile acids were determined by steam distillation of 250 ml. of the culture at pH 2; 750 ml. of distillate were collected and titrated with N/10-NaOH to the phenolphthalein end-point, evaporated and tested for the acids present by paper chromatography using Hiscox & Berridge's technique (11).

CO_2 production

Both media devised by Gibson & Abd-el-Malek (12), and a medium consisting of sterilized milk with citrate added in concentration of 0·5 and 3% (citrate mixture: 1 part of citric acid and 9 parts of potassium citrate) were used.

Growth at pH's 9·6 *and* 9·2

Shattock & Hirsch's (13) medium was used for testing growth at pH's 9·6 and 9·2.

General culture

During the experiments cultures were made in dextrose-Lemco broth every other day and once fortnightly in litmus milk.

RESULTS

Table 1 gives the biochemical and cultural characteristics of the streptococci examined. On the basis of acetoin production the streptococci from the cheese curd fell into four groups. For the sake of brevity only one or two representatives of each group are recorded in Table 1; the third and fourth columns give the number and percentage of all organisms examined. Type strains of *Str. lactis*, *Str. cremoris*, *Str. citrophilus* and *Str. diacetilactis* were also included.

Table 2. *Carbohydrate fermentation*

Group	Code no.	Glycerol	Mannitol	Sorbitol	Xylose	Arabinose	Glucose	Sucrose	Lactose	Maltose	Trehalose	Raffinose	Dextrin	Starch	Inulin	Salicin	Aesculin
I	C30 18	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	+
	C30 35	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	+
II	C30 9	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	+
	C30 62	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	+
III	C30 60	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	+
IV	C30 50	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	+
	Type strains																
	Str. lactis O–J	−	+	.	.	.	+	.	+	+	.	.	.	.	.	.	.
	Str. cremoris HP	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−
	Str. diacetilactis 2	−	−	−	−	−	+	−	+	+	+	−	+	−	−	+	+
	Str. citrophilus PRM/1	−	−	−	−	−	+	−	+	+	+	−	+	−	−	+	+

Tables 2–4 give more detailed information about representative strains only. The streptococci which in Table 1 were put into group IV were not further investigated because of their negative response to the V.P. test. One strain (C30 62) was variable in volatile acid, acetoin and diacetyl production. In this case the amount of volatile acidity determined in 1949 (i.e. 2 years before the present investigation) are included in Table 4. Acetoin was not measured at that time. Strains of *Str. lactis* and *Str. cremoris*, which produced little or no acetoin at 30° C., were incubated at 22 and 10° C. and acetoin determined by the Barritt test (Table 5). The quantities of acetoin produced

Table 4. *Total and volatile acidity, acetoin and diacetyl in milk and broth cultures*

Group	Code no.	Milk culture					Broth culture 24 hr.			
		14 days			24 hr.		Lactose		Lactose + citrate	
		Titratable acidity ml. N/10-NaOH per 100 ml.	Volatile acidity ml. N/10-NaOH per 100 ml.		Acetoin, mg./1000 ml.	Diacetyl, mg./1000 ml.	Acetoin, mg./1000 ml.	Diacetyl, mg./1000 ml.	Acetoin, mg./1000 ml.	Diacetyl, mg./1000 ml.
			July 1949	January 1950						
I	C30 18	67·5	.	10·34	412·0	4·20	20·9	0·80	587·0	7·20
	C30 35	69·0	.	11·69	368·0	3·80	33·9	0·80	617·0	5·40
II	C30 9	74·5	.	2·32	112·6	1·19	12·1	0·54	230·0	5·60
	C30 62	74·0	9·7	1·91	133·1	1·30	10·6	0·60	22·1	1·05
III	C30 37	94·0	.	1·68	.	.	.	.	.	.
	C30 60	80·5	.	2·11	14·5	0·40	2·6	0·28	4·8	0·20
	Type strains									
	Str. lactis O–J	87·2	.	2·06	4·8	0·90	0·0	0·0	0·0	0·0
	Str. cremoris HP	76·0	.	1·71	69·0	1·20	16·9	0·25	24·2	0·30
	Str. diacetilactis 2	91·0	.	10·85	294·0	4·20	1·00	0·20	490·0	6·20
	Str. citrophilus PRM/1	96·5	.	11·08	368·0	3·40	0·50	0·20	363·0	4·10

by various cultures in milk, lactose broth and lactose-citrate broth are also shown in Fig. 1. The rough grouping based on a qualitative V.P. test was confirmed by quantitative determinations. A summary of the results is shown in Table 6.

Table 5. *Acetoin production at various temperatures*

Code no.	10° C. Lactose broth	10° C. Lactose + citrate broth	22° C. Lactose broth	22° C. Lactose + citrate broth	30° C. Lactose broth	30° C. Lactose + citrate broth
Type strains						
Str. lactis						
OJ	No growth	No growth	–	–	–	–
H201	–	–	*S+	+	*VS+	+
H214	–	–	–	–	–	–
H240	+	VS+	+	+	S+	+
H299	+	+	+	+	+	+
Rice	Traces	–	Traces	Traces	–	–
M1	–	–	Traces	S+	–	Traces
M3	–	–	S+	+	–	–
M8	–	–	VS+	S+	Traces	VS+
M14	S+	+	VS+	+	–	VS+
92/4	–	–	Traces	Traces	–	–
95/2	+	No growth	Traces	VS+	Traces	Traces
Str. cremoris						
H–P	No growth	No growth	S+	S+	+	+
J	No growth	No growth	–	–	–	–
236/M	Traces	–	Traces	Traces	Traces	Traces
198/1	–	–	Traces	Traces	VS+	S+
RW	Traces	–	VS+	Traces	S+	+
98/34	–	No growth	VS+	No growth 0·5% citrate	+	+
133/5	+	VS+	S+	+	S+	+
98/31	+	No growth	S+	S+	S+	No growth 0·5% citrate
'N' streptococci from Danish starter:						
Nos. 1 to 19	.	.	+	+	.	.

* S = slight. VS = very slight.

Strains belonging to the first two groups were mainly diplococci or formed short chains. They were arginine and maltose positive and generally grew at 40° C. (A few strains, however, failed to grow at 40° C. in glucose-Lemco broth but grew in yeast-glucose-litmus milk.) In this respect they resembled *Str. lactis*.

Group III included organisms which, by general cultural and biochemical tests, were classified as *Str. cremoris* (maltose and arginine negative, not growing at 40° C. or in the presence of 4% NaCl, forming long and twisted chains).

DISCUSSION

The comparative study of aroma-producing group 'N' streptococci shows that some of our strains isolated from cheese curd (group I) resemble *Str. diacetilactis* or *Str. citrophilus* in respect of strong formation of CO_2, acetic acid and C_4-compounds in the presence of citrate but, unlike them, they were able to produce small amounts of acetoin from lactose only (traces were found with *Str. diacetilactis* and *Str. citrophilus*), the feature which had been discussed by some workers in connexion with acetoin formation by strains of *Leuconostoc* spp. (14, 15).

The quantities of acetoin produced from lactose were less than those produced in the presence of citrate. On the other hand, these strains produced less lactic acid in milk, which suggests that part of the lactose was converted to C_4-compounds. The mechanism of acetoin/diacetyl formation is not yet thoroughly understood. Some investigators assign to citric acid the role of hydrogen acceptor (15) in the metabolism of lactose. Others suggest that citric acid is the starting-point in the formation of C_4-compounds (16-18). The fact that no acetoin was produced from lactose under anaerobic conditions would suggest the need for a hydrogen acceptor in this reaction.

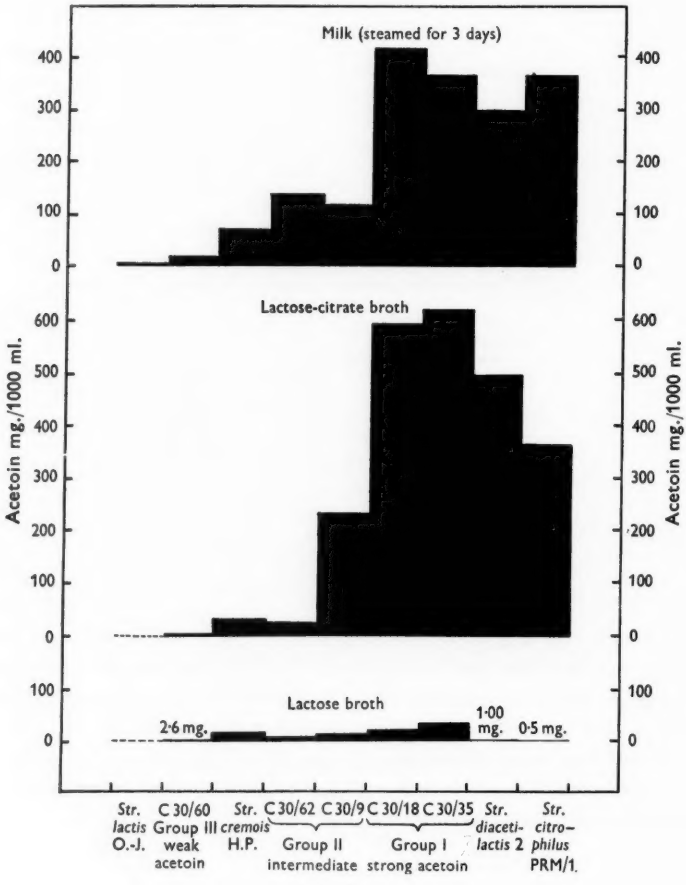

Fig. 1

Table 7. *Variation in the acetoin production December* 1950–*June* 1951

	December 1950		June 1951 (time of distillation)	
Code no.	Lactose	Lactose + citrate	Lactose	Lactose + citrate
C30 9	S+	+	S+	++
C30 62	S+	+	S+	S+

Production of acetoin in small amounts from lactose under aerobic conditions was also observed in strains belonging to group II, as well as in the type strain *Str. cremoris* HP. These strains, however, produced negligible amounts of acetic acid and CO_2 and less acetoin in the presence of citrate than group I streptococci. (As in group I the acetoin was produced under aerobic conditions only.) In some cases there was no significant difference between the quantities of acetoin produced in lactose and lactose citrate medium. Some strains of *Str. lactis* (see Table 5) gave V.P. tests at the lower temperatures in both lactose and lactose-citrate media. The heterofermentation of *Str. lactis* at lower temperatures has already been observed by Bäng (19). It is possible that another adaptive enzymic system develops under other conditions, and may explain this dual fermentation by group 'N' streptococci.

Another feature of streptococci in group II was variability in the production of C_4-compounds. During their period of prolonged cultivation it was observed that one strain (C30 9) became stronger, another (C30 62) weaker in its power to produce acetoin/diacetyl (Table 7). This fact has been previously observed by Swartling (5). In Fig. 1, both *Str. cremoris* HP, and our group II streptococci could be regarded as intermediate acetoin-producing strains at the time of observation. The strain C30 60, though classified as *Str. cremoris*, was less active in acetoin production than the type strains (e.g. HP) and it approached *Str. lactis* O–J in some of its characters.

The relationship of *Str. diacetilactis* and *Str. citrophilus* has been established and the question was raised, whether they deserve a specific name (5). The only differences between our strong acetoin-producing streptococci from cheese and type strains of *Str. diacetilactis* and *Str. citrophilus* are that they produce acetoin from lactose and ferment mannitol.

As already mentioned, the origin of most of the streptococci investigated here was no doubt the starter. Of all the strains isolated, 98% were classified as streptococci of group 'N', 89% had the characters of *Str. lactis* and 9% those of *Str. cremoris*. The *Str. lactis* strains, with the exception of two cultures not coagulating milk, gave a uniform picture in respect of their biochemical and cultural reactions. The only real differences were that they produced lactic and acetic acid, CO_2 and acetoin in varying quantities. The presence amongst them of the 'intermediate' and the strong acetoin-producing strains questions the apparent homogeneity of the group.

The power to produce acetoin from lactose was common to the *Str. lactis* types isolated from the cheese curd. The amounts were similar and the difference between the strains seemed to lie in the degree of citrate utilization, volatile acid production, CO_2 formation and acetoin production. But there were indications that these characters could be acquired or lost in some degree by constant cultivation (strain C30 9 and C30 62), and that there was no clear-cut difference between acetoin-positive and acetoin-negative strains.

Acetoin-producing group 'N' streptococci are not uncommon in starters. A group of streptococci isolated from starter and regarded as *Str. lactis* strains in the N.I.R.D. collection (H201, H240, H299) and homofermentative, 'N' streptococci isolated from Danish starter may serve as an example (Table 5).

It seems quite possible that their role in the cheese-ripening process is something more than the mere formation of lactic acid. The role of diacetyl in flavour of Cheddar cheese has been discussed by some investigators (20,21). Their importance as a source of acetic acid (the only volatile acid found in appreciable quantities in modern Cheddar cheese) may be significant.

SUMMARY

A high proportion of the group 'N' streptococci, isolated from Cheddar cheese curd, produced volatile acids and acetoin, in varying degrees, in milk culture. The active acetoin-producing strains closely resembled type strains of *Str. diacetilactis* (Matuszewski) and *Str. citrophilus* P.R.M. but differed from them in producing acetoin in lactose broth. The weak acetoin-producing strains resembled *Str. cremoris* HP in this characteristic, but in their morphological and cultural reactions, more closely resembled *Str. lactis*. The titratable acidity in milk cultures varied inversely with the production of volatile acids and acetoin.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION D: NUTRITIVE VALUE OF MILK AND MILK PRODUCTS

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I. INTRODUCTION

This review covers some 3 years that have passed since the previous one(1) was completed. With few exceptions, it includes papers published during this time, the wartime arrears having largely disappeared. As before, we have aimed at reading all the accessible papers in the original. Those we have not seen are marked with an asterisk in the bibliography. As in the past the selection of articles was mostly through abstracts published in, or prepared for, *Dairy Science Abstracts*. Readers might like to consult an article(2) explaining the scope and methods of selection of this publication. We are very grateful to Mr A. W. Marsden, Director of the Commonwealth Bureau of Dairy Science, and to his staff, for the invaluable assistance and guidance we have had from them. Our work of collation would clearly not have been possible without the unstinted effort

and hard work of Miss D. Knight, Librarian of the National Institute for Research in Dairying, and her staff, in getting for us all the papers, often not easily accessible.

Though we have attempted to make the review comprehensive we are well aware that many valuable and important papers have been left unmentioned. Even with the facilities of the Bureau and the Library full coverage of the immense literature was an impossible task. To this difficulty must be added our own sins of commission or omission for which we ask indulgence of author and reader alike.

II. GENERAL

The International Dairy Federation has celebrated the fiftieth anniversary of its foundation with the XIIIth International Dairy Congress, the reports of which (3), as usual, contain much information about the nutritive value of milk; individual papers will, as before (1), be dealt with in the appropriate sections. The 1950–1 yearbook of the U.S. Department of Agriculture (4) contains a section, 'Milk: Food for all', with information about the food value of dairy products. A comprehensive review of the nutritive value of liquid milk comes from France (5), and a brief summary of the effect of the diet of the cow on the quality of her milk from America (6). New editions of books (7,8), and a text-book from India (9), devoted partly or entirely to the nutritive aspects of milk, have been published. The *Manual of Nutrition* of the Ministry of Food (10) deals with the place of milk in the diet, and gives a table of nutrient composition of milk. Analytical methods applicable to milk and milk products are described in (11), and tables of food composition (12,13) give values for their major constituents and the better-known vitamins.

The Agricultural Research Council (14) has issued an index of research, including dairying, in progress in Great Britain. Publications from the Commonwealth Bureau of Dairy Science describe briefly research work in progress in the Commonwealth (15,16). More detailed information of research being done comes from Australia (17), Canada (18), the United States (19,20), Holland (21), Scandinavia (22), France, Italy, Portugal and Spain (23), Switzerland (24) and India (25, 26). Publications on dairy research in south-east Asia during the years 1945 to 1949 are listed in (27). A new revised edition of the valuable *Composition of Milks*, published by the (U.S.A.) National Research Council (28), has appeared. Davis (29), in reviewing recent developments in dairy chemistry, includes some information of nutritional interest. Blaxter (30) computed, in terms of human nutritional requirements, the land productivity of vegetable and animal crops including milk.

III. NUTRITIVE VALUE AS ESTIMATED IN LABORATORY AND FIELD EXPERIMENTS

A. RAW MILK (AND BUTTER FOR VITAMINS A, D AND E)

(*a*) *General*

The value of milk as a supplement to cereals has again been demonstrated in rat experiments (31). György (32) found in human milk and other substances, including mucus, a growth factor for *Lactobacillus bifidus*, present only in traces in cow's milk though in appreciable quantities in cow's colostrum. Addition of pig-stomach mucin containing this growth factor increased the growth rate of rats given as sole diet cow's milk supplemented with lactose and fat to simulate human milk (33). Using an unsupplemented milk diet Adams (34) found that copper and manganese, but not iron, were involved in the maintenance of normal liver-catalase activity in mice. The growth-promoting value of mineralized milk for rats was not increased by the addition of vitamins of the B complex (35). Fawns & Bourne (36,37) found that, although milk with fruit syrups added produced finer clots in the stomach of rats than milk alone (36), the growth rate or nitrogen absorption were not thereby improved (37). Abbott, French & Townsend (38), with milk from two different sources, observed marked differences in growth and well-being of rats given diets of two-thirds wheat and one-third milk calculated on the dry basis. They give no information about the composition of the milks; the interpretation of their findings is impossible. In

rats exclusive diets of mineralized milk produced scaly dermatitis of the paws exacerbated by low relative humidity and prevented by addition of liver, beef muscle or non-essential amino-acids (39, 40) (see also p. 258).

The knowledge about relation of diet to resistance to infection is still very confused. Addition of skim milk to the rations of chicks increased their resistance to infection with *Ascaridia galli* (41). In the critical experiments of Howie & Porter (42) addition of milk to a diet improved reproduction and growth in mice, but lowered their powers of resistance to infection with tubercle bacilli or Salmonella organisms. Diets of fresh or reconstituted milk, supplemented with thiamine, pyridoxin and pantothenic acid largely suppressed experimental *Plasmodium berghei* malaria in rats (43). In this instance growth was better but resistance less with a stock diet containing much less milk. It does not, therefore, follow that a diet enhanced in certain respects is necessarily so in others. Hawking (43*a*) ascribes the suppressive action of milk to its deficiency in *p*-aminobenzoic acid, essential for the growth of the plasmodium.

Blaxter (44) reviews briefly the evidence against the passage of the thyroid hormone into milk. Edwards & Haskins (45) observed that, although antibiotics injected intramuscularly into cows persisted longer in their milk, the concentration was less than after intravenous injection; results varied with the antibiotic used. The high concentration of penicillin in milk, resulting from the treatment of mastitis, that interferes in the subsequent manufacture of dairy products, notably cheese, and the means of overcoming this interference are discussed in (46).

The use of skim milk in pig rations is discussed in (47, 48, 49, 50), and its value in baking bread from frozen doughs in (51).

(*b*) *Vitamins*

(i) *General*

The first volume of a book on analytical methods for the dairy laboratory (11) contains a chapter on the vitamins of milk with a comprehensive table giving values, together with brief summaries of the methods of determination; methods for some of the better known vitamins are described in greater detail. A chapter on vitamins in milk with some information on their content under Russian conditions of husbandry is included in (52). A compilation of values for the known vitamins in milk and milk products is given in (28, 53).

Chemical and microbiological methods for vitamin assays used for milk at Shinfield are described in (54), where values are quoted for seven B-complex vitamins, carotene, vitamin A and vitamin C in bulk milk from the Institute herd. A further paper from Shinfield (55) presents in detail biological and microbiological methods used for the measurements of vitamins of the B complex and reflects good agreement between the two kinds of method for riboflavin and pantothenic acid in milk. In a similar comparison of methods in Cambridge (56) good agreement was found with milk between chemical and microbiological tests for riboflavin and nicotinic acid, and between chemical and biological methods for thiamine. Kon (57) discusses factors affecting the vitamin content of milk, mainly those studied at Shinfield. Ritter (58) discusses in a general article the use of micro-organisms for the determination of vitamins and amino-acids in milk and its products, and reviews briefly microbial vitamin synthesis in the fermentation of milk and ripening of cheese. Values for a number of vitamins in milk and milk products are listed in (59, 60, 61). The effect of feeding iodinated protein to cows on the concentration of ascorbic acid, thiamine, riboflavin and nicotinic acid in milk is briefly reviewed by Blaxter (44). An anti-peptic ulcer factor (vitamin U) is said to be present in milk and butter (62).

(ii) *Vitamin A and carotene*

Deuel's (63) comprehensive treatise on lipids and Goodwin's (64) book on carotenoids contain information about vitamin A and carotene in milk and colostrum (see also (65)). Paper (66) reviews some of the literature on relationships between carotene in the feed and carotene and vitamin A in blood, colostrum and milk, and reports *in vitro* experiments indicating intestinal conversion of carotene in the bovine.

Methods of measurement. Several laboratories have reported their routine methods for the determination of vitamin A and carotenoids in milk (54, 67, 68, 69, 70, 71, 72, 73). The method for the

direct estimation of vitamin A in butterfat by the antimony-trichloride reaction described in (74) seems to be based on quite unsound principles. Papers (68) and (69) describe the methods used for the measurements of the alcohol and ester forms of vitamin A, and of β-carotene in total carotenoids. A brief statement in (75) indicates lower values for vitamin A activity of butter by chemical test than by biological assay. The biological assay of vitamin A activity in milk is given in (76).

Colostrum. Cordiez (77) has published a review of work on the vitamin A and carotene contents of cow's colostrum, with special reference to the significance of these factors in the nutrition of the calf. The rapid fall in the concentration of vitamin A and carotene in colostrum with the progress of lactation has again been demonstrated with colostrum (67) and colostral fat (68) in which the decline was logarithmic (cf. (1)). Chanda's work (68) lends statistical significance to the observations that the colostrum of heifers contains more of these factors than that of cows (cf. (1)). According to him, β-carotene constituted some 20–25% of the total vitamin A activity of colostrum, both from cows on pasture and those on winter feed. Hennaux, Antoine & Lecomte (67), on the other hand, found no such constant relationship, carotene in their experience being higher and vitamin A lower in summer than in winter.

In the experiments of Stallcup & Herman (66) heifers given Korean lespedeza [*Lespedeza stipulacea*] hay for 8 weeks before calving produced colostrum richer in vitamin A and carotene than that similarly obtained with lucerne hay that had much less carotene. On both, the levels were low and, surprisingly, in three of the six animals vitamin A and carotene were more concentrated in the second milking than in the first. In fact, values for the first colostrum are, in one or two instances, lower than in later milk, and the values for successive milkings fluctuate to an unusual extent; it is not stated whether fluctuations in fat content influenced these findings.

According to Goodwin & Wilson (78), 94% of the vitamin A of colostral fat is in the ester form. Chanda (68) found the same value for cows but 90% in heifers.

Prepartum milking continues to claim attention. In a large-scale experiment Hill, Widdowson & Maggs (79) found a mean concentration of carotene at parturition of 4·3 mg./l. for pre-milked cows and of 8·7 for those not so treated. In experiments at Shinfield (80), with a group of eight cows pre-milked for 14 days, vitamin A and carotenoid concentrations rose to a peak a week before calving and thereafter fell steeply; little change in concentration was observed at parturition. Indian workers (81), on the other hand, with a group of thirteen cows noted very marked variations during 10 days prepartum, but the mean figures indicated a definite increase in concentration of both vitamin A and carotene immediately after parturition. The difference between the two sets of findings might well have been due to differences in yield of the prepartum secretion (cf. (80)). Though Goodwin & Wilson (78) state that the concentrations of these factors in the secretion obtained 2 and 6 days prepartum were as high as at calving, their values are for different cows and therefore not comparable.

In the first milking of colostrum of the Brown Latvian breed Valdman (82) found 3200 i.u./100 ml. vitamin A activity in summer and 680 i.u. in winter. Addition of carrot silage and good hay, supplying from 1 to 1½ million i.u. carotene daily to the ration of dairy cows, definitely increased the vitamin A activity of their colostrum; a vitamin A concentrate was even more effective (82). Daily doses of some 500,000 i.u. vitamin A for up to 80 days before calving increased the vitamin A activity of the colostrum by some 25% and equivalent doses of carotene rather less (83). Kantov (84), in Czechoslovakia, quotes the astonishingly high value of 3500 i.u. vitamin A activity per g. colostral fat. Hibbs, Pounden & Krauss (85), in continuation of earlier work (86), confirmed that large doses of vitamin D (5 million units daily for 2 weeks) did not influence the vitamin A or carotene content of the colostrum of normal cows or of cows suffering from milk fever. Storage of colostrum at −5° F. for 6 months caused no loss of vitamin A and less than 6% loss of carotene (87). Detailed values for vitamin A in the colostrum of two herds of Murrah buffaloes in India are given in (88). The mean concentration in the first samples of colostrum were 118 and 203 i.u./g. fat, the difference being attributed to differences in feeding and management.

Milk. Reports on the seasonal variation of the vitamin A and carotene content of milk are available for Holland (89,90,91), Germany (92,93,94,95), Poland (96), Latvia (82), Azerbaijan (97), the United States (98,99,100), Mexico (101), India, both for cows and for buffaloes (vitamin A only) (102), and New Zealand (70,71). In the northern hemisphere the usual peaks in the concentration of these nutrients were observed during the early summer and often also in autumn. In New Zealand (70,71), on the other hand, previous findings (cf. (1)) that the peak occurs in winter have been confirmed in extensive experiments covering 3 years. Samples were collected at fortnightly intervals from twenty factories in five main districts. In four districts in the North Island maximum and minimum concentrations were observed in July and between November and March each year. In the South Island (single district) the seasonal changes were less marked, and values more variable, but maximum values occurred during the winter. With cows all the year at pasture, supplemented when necessary with pasture silage and green-pasture hay, there is in New Zealand little seasonal variation in the supply of carotene in the feed (70,71,103). The winter peak must, therefore, be due to factors other than changes in carotene intake.

In experiments with monozygous twin calves McGillivray (103) has shown that the carotene in summer pasture is more efficiently absorbed with a supplement of vitamin E. New Zealand summer pasture contains a large proportion of clover, and this is considerably lower in vitamin E than ryegrass which predominates in winter (103). Under different conditions, Krukovsky, Whiting & Loosli (104) with Friesian, Brown Swiss, Jersey and Guernsey cows observed a correlation between tocopherol and carotene but not vitamin A. McGillivray (103) found that in butterfat vitamin A potency and vitamin E content are closely correlated. The observation on the influence of vitamin E may go far to explain some discrepancies between the carotene content of forages and their effect on the vitamin A potency of the milk observed in the past (see e.g. (105)).

The effect of the carotene content of the feed on the vitamin A activity of milk continues to claim attention (66,91,93,97,98,104,106,107,108,109,110,111,112,113). Stallcup & Herman (66) emphasize the closer relationship between carotene of the feed and milk carotenoids than with the vitamin A, and Dutch workers (91) make similar observations. There was no correlation between the vitamin A and carotene content of the milk and environmental temperature (50–105° F.), except in so far as the consumption of hay decreased at the higher temperature (66). Differences in the carotene content of butter obtained in different districts of Holland (91) are attributed to differences in the feeding of the cows, e.g. the more extensive use of artificially dried grass in Friesland than in other provinces. The vitamin A activity of winter milk was notably low in two herds in the Paris area (109). The better preservation of carotene in silage than in hay made from the same pasture was reflected in the higher concentration of carotene and vitamin A in the milk (108). Silage with its pH adjusted to near neutrality was rather better in this respect than untreated silage (112). Raw soya beans are known to depress the vitamin A and carotene content of milk (cf. (1)). They can be given, however, to the extent of 20% of the ration with liberal quantities of hay without exerting this effect (114). Replacement of oats in the ration by germinated oats decreased the vitamin A activity of milk (115).

According to Valdman (82), 500,000 i.u. vitamin A given daily to cows as a concentrate increased the vitamin A activity of milk to 260 i.u./100 ml. as against 113 i.u. in the milk of control cows; surprisingly, with a dose 10 times as big the potency of the milk was no higher. 8 kg. daily of carrot silage containing 1 million i.u. carotene gave, in the same experiment, milk with 230 i.u./100 ml. The effect of feeding carotene as palm-oil to stall-fed cows on the carotene and vitamin A content of their milk is reported in (116,117,118); quantities up to 1 g. daily had little effect (116,117,118), but 2 g. raised the potency to some 75% of that obtained with the same cows on pasture (118), and 14 g. gave values typical of summer butter (117). Vitamin A in aqueous dispersion injected intravenously into cows at the rate of 230 μg./lb. body weight was more rapidly and more efficiently transferred to milk than when given in the same form by mouth, and this in turn was more efficient than when given in oily solution (119). The modest dose of 40,000 i.u. (U.S.P. units) of vitamin A given daily for 4 months to cows on unspecified rations increased

significantly the vitamin A content of their milk but did not depress the carotene; with other cows a daily supplement of 140,000 units depressed the carotene (120).

From India (121) comes the report that shark-liver oil, supplying 100,000 i.u. vitamin A daily, increased within 24 hr. the vitamin A concentration of milk fat of cows from 13 to 51 i.u./g. and of buffaloes from 14 to 45 i.u./g. In both species the concentration fell during the ensuing 6 days but rose again while the dose was doubled; in cows the carotene content of the milk fat decreased during the administration of the shark-liver oil.

Methods of increasing the yellow colour of butter are described in (93, 122, 123, 124, 125). In a 3-year experiment the daily addition of 10–20 kg. of carrots to the winter feed of cows increased the colour of the butter to summer levels (93). Direct addition of carotene to the butter is recommended in (122), (123) describes the chromatographic detection and separation in butter or margarine of carotene, anatto and butter yellow, (124) gives a method for measurement of natural or added colour in the standardization of the colour of butter, and (125) the preparation of dye tablets.

No change in the vitamin A and carotene concentration of milk followed the giving to cows of three samples of commercial iodinated protein (126) (cf. (1)). Chanda & Owen (113) found more complicated relationships after administration of thyroxine or thiouracil. The proportion of the alcohol form of vitamin A in milk increased markedly when thyroxine (113) or thyrotrophin (127) were administered with a carotene-free diet. In normal milk the alcohol form of vitamin A accounted for only some 5% of the total (113, 128).

According to Chanda (68), the carotene content of milk remained steady after the first 3–4 weeks after parturition, but that of vitamin A decreased slowly throughout lactation to rise again towards its end. It is not clear, however, from the paper whether and to what extent these lactational trends were influenced by seasonal changes in feeding. In mastitis the concentration of β-carotene increased in milk from infected quarters, the vitamin A concentration remaining constant, but there was an increase in the alcohol form depending on the degree of mastitis (128).

Breed effects on the vitamin A and carotene content of milk are discussed in (102, 107).

Butter stored at low temperatures (mostly 0–14° F.) for periods up to 180 days lost little or none of its vitamin A activity (53, 70, 71, 99, 129, 130, 131). Where loss occurred it was in vitamin A rather than carotene (99, 130) and was associated with loss of flavour (130, 132). According to (53) carotene in butterfat was more stable at -5 to $+2$° C. than at -18 to -10° C. as at the lower temperatures crystallization of higher melting-point glycerides increased the concentration of carotene in the unsaturated fraction where it was more subject to oxidation. A brief summary of work at Iowa on the effects of storage on the vitamin A potency of butter is given in (75). A mixture of α-tocopherol and ascorbyl palmitate proved effective in reducing loss of carotene in accelerated oxidation tests with butterfat (132). The effect of methods of manufacture of ghee or 'samna' (clarified Egyptian buffalo-milk fat) on the survival of vitamin A (and carotene) are discussed in (133, 134), and stability during storage in (133, 135). Losses of vitamin A in ghee when used for cooking are described in (136).

(iii) *The vitamin B complex*

A microbiological method applicable to milk for measurement of thiamine, riboflavin, nicotinic acid, pantothenic acid, vitamin B_6 and biotin is described in (137) where single values are quoted, and paper (138) gives microbiological methods for riboflavin, nicotinic acid, pantothenic acid and biotin. (See also (54).)

Thiamine (*vitamin* B_1). Bouman (139) found it necessary to introduce in the fluorimetric determination of thiamine in milk adsorption on Franconite KL after Taka-diastase digestion, otherwise spuriously high values were obtained. Measurement with *Phycomyces blakesleeanus* after Taka-diastase gave normal values.

A thiochrome method involving, after pepsin treatment, incubation of milk with *Aspergillus* is described in (140). Davidov & Gulko (141) describe a thiochrome method in which thiamine is liberated by acid hydrolysis followed by incubation with penicillin felt and by Decalso treatment.

For the colostrum of three Sindhi cows, Rao & Basu (142) found maximum values of 130–180 μg./100 ml., and for one Murrah buffalo of 170 μg./100 ml. Davidov & Gulko (141, 143) found no obvious effects of stage of lactation or of season on the thiamine content of milk and report normal (mean) values of around 40 μg./100 ml. whole milk. Values for thiamine in the milk of Sindhi, Gir, Tharparkar, Sahiwal and crossbred cows and of Murrah buffaloes are given in (142). Mean values are within the range 45 μg./100 ml. (crossbreeds) to 54 (Gir), the breed difference being statistically significant. As expected, high values were observed at the beginning of lactation and there was also a terminal rise. Seasonal fluctuations were irregular. Wagner (95) quotes incredibly low values of 10–16 μg./100 ml. for raw milk in Germany from January to April, but normal ones thereafter.

The partition of free and bound thiamine was studied in cow's milk as influenced by stage of lactation (144), injection of thyroxine or thiouracil (145), or mastitis (128). In all of these studies a negative correlation was found between the phosphatase content of the milk and the protein-bound thiamine and co-carboxylase. Total thiamine decreased during lactation (144) and also, slightly but significantly, as a result of thiouracil injections (145), and markedly during mastitis (128); it was not affected by thyroxine injections (145). The concentration of co-carboxylase and of protein-bound thiamine decreased in mastitis (128). Thyroxine markedly increased the co-carboxylase and slightly, but significantly, the protein-bound thiamine; with thiouracil the reverse held (145).

Methods for the removal by means of Florisil of most of the thiamine in milk used in experimental diets are described in (146, 147).

Riboflavin. Fluorimetric methods for the determination of riboflavin applicable to milk are described in (54, 141, 148, 149) and microbiological methods in (54, 150). In the last paper a discussion of different methods will be found. Paper (147) deals, among other problems, with reasons for the discrepancy between fluorimetric and microbiological tests of riboflavin in milk, and ascribes lower values obtained by the former method to incomplete extraction of the combined form of riboflavin.

Maximum values for riboflavin in the colostrum of three Sindhi cows were from 210 to 360 μg./100 ml., and for one Murrah buffalo 320 μg./100 ml. (142). Values for whole milk are given in (54, 95, 138, 141, 142, 143, 148, 149, 151, 152, 153, 154, 155). With the exception of those in (95, 141, 143) they fall within the range 120–225 μg./100 ml., the high value being for bulk milk, probably containing a high proportion of Jersey milk (152) or for milk of Jersey cows (153). From Russia (141, 143) low values, 75–100 μg./100 ml., are reported from one farm, but a much higher value, 160 μg./100 ml., is given for the Moscow milk supply.

Of the Indian breeds mentioned above Sindhi milk with 170 μg./100 ml. was significantly higher in riboflavin than the other milks (130–160 μg./100 ml.). The buffalo milk contained 107 μg./100 ml. (142). In the United States, Jersey milk was, as usual, richer than Friesian (233 against 170 μg./100 ml.) (153). Wagner (95) claims to have detected in German milk only 15–22 μg./100 ml. in January to April, and 70–175 μg./100 ml. thereafter. The low values are hardly credible. Hoeflake (138) found higher values in Dutch bulk milk in summer (around 200 μg./100 ml.) than in winter (around 140 μg./100 ml.), the summer rise coinciding with pasture feeding. In southern England somewhat higher values were observed in July–December than during the rest of the year (155). In Italy (156, also in 157) values were low, but higher in summer than in winter, 115 μg./100 ml. compared with 83 μg./100 ml. In Russia (141, 143), however, the content was rather higher during stall feeding than on pasture. Higher values for winter milk are also reported from India (142), where they may have been connected with winter calvings, and from the United States (154). Confirmation of the established fact that mastitis lowers the riboflavin in milk will be found in (153). The breed differences noted in this paper persisted in the mastitis samples. Injections of thyroxine or of thiouracil did not affect the riboflavin content of milk (145).

A fuller report of the English study on the destruction of riboflavin in milk between bottling and consumption has now been published (155). Under normal conditions of delivery in covered vans, mean losses amounted to 4·1–8·0%, though considerable individual variations occurred.

With bottles intentionally exposed to light during the delivery round the losses, depending on the amount of sunshine, were about 20% in the spring and about 50% during the summer months. Protection against loss of riboflavin afforded by the use of coloured bottles is discussed in (158,159). Milk distributed by the Moscow dairy combine contained some 15% less riboflavin than the original supply. It is not stated whether these losses in handling and distribution included those in any heat treatment (143).

Methods for the removal of riboflavin from milk are based either on treatment with Florisil (146,147) or on ultraviolet irradiation (147,160). The relationship between the riboflavin content and the redox potential of milk is discussed in (161).

Nicotinic acid. In addition to methods mentioned under '*General*', p. 247, a chemical (141) and a microbiological method (138) are described for the assay of nicotinic acid. The colostrum of a Sindhi cow had a maximum of 71 μg./100 ml. and that of a Murrah buffalo of 63 μg./100 ml. (142). In (162) values of 70–110 μg./100 ml. fresh milk, according to the method of extraction, are given. A value of 73 μg./100 ml. is reported from Denmark (137). Much higher values (around 150 μg./100 ml.) were obtained in Russia by a chemical method (141) (see also (143)). The content was rather higher during stall feeding than on pasture, and did not vary with the stage of lactation (141). In the study of different breeds in India (142) (see p. 251) no breed differences or marked seasonal changes were noticed, the values were around 80 μg./100 ml. In Holland the content fluctuated between 70 and 100 μg./100 ml. with a peak at the beginning of the pasture period (138). From Finland low values, about 35 μg./100 ml., are reported for two cows; they increased rapidly within 24 hr. after a single dose by mouth of 5 or 10 g. nicotinic acid (163).

Pantothenic acid. A microbiological method of assay is described in (164). Values for the pantothenic acid content of milk are given in (54,137,138,164); they fall within the range of 200–400 μg./100 ml.

Pyridoxin (*vitamin* B_6). The use of the mould *Neurospora sitophila* for the assay of the vitamin B_6 complex is described in (165,166). According to (165) thiamine must first be removed, but Debrit (166) thinks that in quantities normally present in milk it does not interfere with the measurement. He found, however, that the method is only one-tenth as sensitive as that using the yeast *Saccharomyces carlsbergensis*. He quotes only values by the *Neurospora* method and these are high, 100–200 μg./100 ml. (cf. (1), (167)). That of 650 μg./100 g. fresh milk (165) is perhaps a misprint, as a value of 700 μg./100 g. is quoted in the same paper for whole dried milk. A value of 57 μg./100 ml. comes from Denmark (137) and one of 37 μg./100 ml. from England (54). The destructive effect of sunlight on vitamin B_6 in milk is noted in (165,166) and that of ultraviolet irradiation in (160,165).

Biotin. Values of about 2 μg. biotin/100 ml. milk are given in (54,137,138). No effect of season was found in Holland (138).

Choline. A value of 51 mg./100 ml. is quoted in (82) for colostrum and one of 6 mg./100 ml. for milk.

Folic acid. According to (168) (see also (169)) first-day colostrum of Friesian and Guernsey cows contained in 100 ml. 0·9–5·0 μg. folid acid. On the third day values normal for milk were found. The assay of folic acid with *Streptococcus faecalis* is described by Jones & Morris (170), who detected no more than traces of this vitamin in milk. Using the same organism the Wisconsin workers (168,169) found in the milk of some twenty Friesian, Guernsey and Jersey cows 0·1–0·4 μg. free folic acid/100 ml. and observed no breed differences or marked lactational trends. About 20% of the folic acid activity of milk was due to folinic acid (168). Growth and haemoglobin formation in young rats were not improved by the addition of folic acid to a mineralized-milk diet (171).

Cyanocobalamin (*vitamin* B_{12}). In certain natural materials, though not in milk, vitamin B_{12}-like substances are present, active for assay micro-organisms but not for higher animals (cf. (172)). A microbiological method specific for cobalamin has just been published (173). Conditions for the preparation of milk for the U.S.P. microbiological method of vitamin B_{12} assay are discussed in (174).

Colostrum of American cows contains, according to (168, 169), some 1–2 μg./100 ml., but much higher values, about 2–5 μg./100 ml., have been reported from another laboratory (175). As pointed out below, differences in technique may be involved. For colostrum of Indian cows a preliminary value of some 0·3 μg./100 ml. is suggested (176). Jersey colostrum was poorer in vitamin B_{12} than that of Friesians (168, 175) or of Guernseys (168).

Papers (177, 178) deal with the form in which cyanocobalamin occurs in milk. Both laboratories quote values of 0·3–0·4 μg./100 ml. (see also (54)), and a value of 'about' 0·5 μg./100 ml. is given in (179). Mean values for American market milks from large numbers of herds are also about 0·3–0·4 μg./100 ml. (168). Values quoted in an earlier publication (169) are rather higher. Much lower values, about 0·1 μg./100 ml., come from India for Sindhi, Tharparkar and Gir cows (176), and higher values of 0·6–1·0 μg./100 ml. from Holland (180). The differences may well be partly due to differences in technique of measurement. Most, if not all, of these values were obtained by microbiological measurements. By rat assay Hartman & Dryden (181) obtained the higher value of 0·8 μg./100 ml.

No significant breed differences have been observed in the vitamin B_{12} content of milk. All laboratories report very marked individual variations.

Supplements of vitamin B_{12} (182, 183, 184) or of cobalt (181) (see also (168)) given to cows did not increase the vitamin B_{12} content of their milk. Koetsveld (180), with a group of ten Friesian cows, noted an increase from 0·6 to 1·1 μg. vitamin B_{12}/100 ml. on changing from stall-feeding to pasture, and somewhat higher concentrations in evening than in the morning milk, but the reverse has also been reported (175).

The addition of vitamin B_{12} to a mineralized-milk diet did not affect growth and haemoglobin formation in the rat (171). The importance of milk as a source of vitamin B_{12} is stressed in (185). Schweigert (186), in reviewing the nutritional significance of vitamin B_{12}, points out that it can replace 'nutrient X' and zoopherin but not the growth anti-anaemic factors needed by the fox and mink, and present in milk.

Other factors. Isolation of orotic acid from milk is described in (187); its concentration, which varies from 5 to 10 mg./100 ml. milk, is not affected by breed or season. The presence in milk of vitamin B_T, a growth factor necessary for the growth of meal-worm larvae and identified as carnitine (188) has been demonstrated (189). It is found in the whey and not in the casein fraction of milk. Milk is said to contain a growth factor necessary for the rat (190). Xanthopterin oxidase (191) and xanthine oxidase (192) have been isolated from milk. It contains a factor involved in xanthine-oxidase activity (193).

(iv) *Ascorbic acid (vitamin C)*

A modification of the indophenol-titration method is described in (194), and a method for the determination of ascorbic, dehydroascorbic and ketogulonic acids in milk with 2 : 4-dinitrophenylhydrazine is given in (195).

Values for raw or unspecified milk will be found in (82, 94, 107, 108, 154, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203). Some of them (154, 196, 198, 202, 203) definitely represent only the reduced ascorbic acid, and with some the experimental details are lacking, but on the whole normal values about 2 mg./100 ml. are quoted. Paper (200) gives much higher values of 3–3·8 mg./100 ml., obtained by the method of Roe & Kuether, than those usually believed to be characteristic of freshly drawn milk. Wagner's values (95) for German milk can only be described as derisory. He found no vitamin C at all in January to March but up to 9 mg./100 ml. in the summer months.

Paper (154) deals with fluctuations in reduced ascorbic acid in Ohio market milk, (201) with the losses, presumably also in the reduced form, during storage of milk on the farm and transport to processing plant, and (203) compares milk at the retailer with that in the home. Keeping experiments with the milk of one cow are reported in (203). According to Larson & Jenness (204), ascorbic acid is the main dialysable reducing substance in milk.

The relationship between oxidized flavour and ascorbic acid in milk continues to attract attention; as in the previous review (1) work on both raw and heat-treated milk will be considered

together. A review on oxidized flavours by Kruisheer (205) deals with the literature and sums up present knowledge about the part of vitamin C in their formation. Papers (206, 207, 208, 209, 210, 211) deal with the effect of additions of ascorbic acid, of copper or of both on the development of the flavour, and paper (212) with their effect in conjunction with exposure to sunlight. Small quantities of ascorbic acid, with or without copper, enhanced development of the flavour (207, 209, 211, 212) (cf. (1)). Substantially larger quantities of ascorbic acid gave protection (208, 211). Krukovsky (209) confirmed the previous findings (cf. (1)) that complete removal of ascorbic acid increased the resistance of milk to the formation of the copper-induced flavour, more so when the ascorbic acid was destroyed by H_2O_2 than when destroyed by exposure to light and subsequent heating. The susceptibility of milk to the development of this fault varied with the individuality of the cow (213) and the type and quality of the roughages given to the cows (210). Giving propylgallate to cows had no effect on the susceptibility of their milk to the taint (213). During prolonged storage of milk development of the flavour was associated with loss of ascorbic acid (212). The influence of additions of ascorbic acid on the development of the oxidized flavour in cream is discussed in (214, 215, 216). Ascorbic acid proved unsatisfactory as an anti-oxidant in butter (217, 218).

The destruction of vitamin C by light is discussed directly in (209, 212, 219), and by implication in (202, 203). The use of coloured bottles in preventing this loss is considered in (158, 159). Géro (219), apparently unaware of the photochemical oxidation of vitamin C in milk induced by riboflavin, wrongly ascribes the effect of light to the catalytic activity of copper ions.

In the experience of Chanda, McNaught & Owen (145) thyroxine injected into cows lowered the ascorbic acid content of milk; they therefore deny that similar effects of iodinated protein might have been due to free iodine rather than to the hormone (cf. (1)). Injection of thiouracil had the opposite effect (145). Papers (220, 221) deal with the relation of ascorbic acid to the peroxidase of milk, paper (222) with the oxidation of ascorbic acid in milk by hydrogen peroxide, and paper (223) with its part in the catalytic oxidation in milk of various added aldehydes.

(v) *Vitamin D*

General information about the vitamin D content of milk is given in (63). The tentative biological method of the Association of Official Agricultural Chemists (cf. (1)) has now been adopted officially (224); the biological method used at Shinfield is described in (69). Values of 20 i.u. vitamin D/100 ml. colostrum and 3 i.u./100 ml. milk are reported from Latvia (82). An impossibly high value of 400 i.u./g. colostral fat is reported from Czechoslovakia (84). The high values for the vitamin D potency of Indian butters, commented on in the previous review (1), have not been confirmed, and papers (225, 226) quote values of some 0·3–1·0 i.u./g. fat, i.e. of the same order as in summer butter in temperate climates. In German milk Wagner (227) detected no vitamin D between October and May and 20–40 i.u./l. in May–September with the peak in July.

(vi) *Tocopherol* (*vitamin E*)

Information about tocopherol and its concentration in milk is given in (63). A method for the determination of tocopherols in milk in the presence of oxidized fat is given by Handwerk (228), who thinks that the γ- and δ-isomers accompany, in milk, the α-form only in faint traces. Brown (229) describes a paper-chromatographic method for separation of α-, β-, γ-, and δ-tocopherols; he too found only α-tocopherol in the milk of cows on pasture. On the same assumption, Kieferle, Seuss & Schraudy (230) describe a modification of the Emmerie & Engel colorimetric method.

Colostrum contains more tocopherol than milk (cf. (1)). Thus Kieferle *et al.* (230) found in the colostrum of two German Brown Mountain cows 300–400 μg./100 ml. Valdman (82) gives values of 300 μg./100 ml. in Latvian winter colostrum and 1000 μg./100 ml. in summer, but the German workers observed no seasonal differences. By contrast, Wagner (95), also in Germany, claims to have detected marked seasonal variations. His values for the winter months are only about

25 μg./100 ml.; in summer he finds around 60 μg./100 ml. The figure of 900 i.u. (i.e. mg.) per g. colostral fat quoted by Kantov (84) from Czechoslovakia is obviously an error or a misprint.

In summer milk, Valdman (82) found 200 μg./100 ml. and half this concentration in winter. The German values for bulk milk are 87 μg./100 ml. in winter and 96 μg./100 ml. in summer, the difference representing differences in fat content as the concentration in fat (24 and 25 μg./g.) did not vary (230). The mean content of fifteen samples of commercial butter in Iowa was 29 μg./g. (228) and similar values were found in Norway (107, 108).

The effect of the feed of the cows on the tocopherol content of milk has been reviewed in (231). Experiments in Norway (107, 108) showed appreciable differences in the tocopherol content of milk with different fodders, and from Belgium (115) comes the unexpected observation of a marked decline in the tocopherol content of milk when cows were changed from ungerminated to germinated oats. American workers (104, 232) observed a gradual decline in the vitamin with cows on a winter ration, and a definite rise when they went out to pasture. Addition of 1–2 g. daily of mixed tocopherols raised the vitamin in the milk at the beginning but not towards the end of the winter feeding (232). Interesting observations on the relation of the tocopherol content of milk to stage of oestrous cycle, fat content and stage of lactation, and its variation in milk from individual quarters and from successive partial milkings will be found in (230).

Breed differences are reported in (104); the lowest mean concentrations throughout the year were found for Friesians (22 μg./g. fat) and the highest for Guernseys (30 μg./g. fat).

The relationship between tocopherol content of milk and development of oxidized flavour is dealt with in (104, 107, 108, 183, 205, 209, 210, 216, 232, 233). The bulk of the evidence shows that resistance to oxidized flavour is associated with higher tocopherol concentrations (107, 108, 209, 210, 232, 233) and Krukovski, Loosli & Whiting (233) found a highly significant correlation in this respect. Addition of 10 mg. tocopherols/kg. butter had no effect on the keeping quality with or without the addition of ascorbic acid (183, 216). Hove & Harris (234) found a highly significant correlation between the tocopherol and linoleic acid content of a number of animal and vegetable fats including that of milk.

(i) *Minerals* (c) *Other factors*

A rapid method for the determination of calcium in milk is described in (235), and one requiring only small quantities of biological materials in (236); the former gave low values for milk calcium, but with the latter there was satisfactory agreement with the normal McCrudden technique. Breyer & McPhillips (237) describe an indirect polarographic method for the determination of calcium in the presence of magnesium. For milk it has an accuracy of ±3%. A method for studying the distribution of calcium, magnesium and phosphate in skim milk is summarized in (238). Representative values for the calcium in milk are available for England (60), Canada (239) and Japan (240). Seasonal variations in calcium and phosphorus content are reported in (241, 242) and are deduced in (243). The calcium content of milk was not affected by milk fever (85, 244). In Germany, Rauschning (245), using his own method, found in milk from eastern Thuringia a higher calcium content (0·17%) than that (0·11%) quoted by him from other workers for eastern and northern districts; in Mississippi (241) the calcium content of milk was not affected by variations in the concentration of this element in the soil in different areas. Increase in the pH of silage by addition of Na_2CO_3 is said to have lowered significantly the calcium content of milk (112).

The method of transfer of phosphorus (246) and of calcium (247, 248) from feed to milk has been studied with the aid of radioactive isotopes. Chanda & Owen studied the partition of phosphorus in normal milk (144), and in the milk of cows given injections of thiouracil or thyroxine (249). They ascribed the decrease in total phosphorus after thiouracil and increase after thyroxine injections to changes in the ester and lipid phosphorus (249). The calcium content was not affected by injection of either substance (145).

Methods for the determination of iron in milk or cream are given in (250, 251, 252, 253); that described in (251) is for the determination of both iron and copper in the one sample. Feuillen &

Plumier (254) quote a value of 45 μg./100 ml. for milk taken directly from the udder, and Lederer (255) records values for the iron content of some milk products. Mulder & Koppejan (256) have studied the distribution of iron and copper in various phases of milk, about 25 % of the iron of whole milk was bound to the fat globules. The effect of its iron content on the keeping quality of butter is discussed in (257,258,259,260,261,262); the degree of oxidized flavour was higher in samples with a high iron content and the flavour did not occur in butters containing less than 500 μg./kg. (257,259), i.e. in butters not contaminated with adventitious iron. Marked iron contamination is reported for butter produced in low-ranking factories in the Netherlands (263). According to (260) the catalytic effects of the contamination depend not so much on its absolute quantity as on its presence in the finely dispersed droplets of buttermilk in the butter.

Methods for the determination of copper in milk and milk products are given in (251,252,253, 264,265); a micro-method for the detection of copper in foods, including milk, will be found in (266). Papers (267,268) confirm that the copper content of milk is not influenced by the feed of the cow. Koppejan & Mulder (264), studying the copper content of milk of four cows during the first 60 days of lactation, noted an increase during the first 2–6 days, followed by a gradual return to normal levels; the variations between individual cows were marked. Munch-Petersen (267) also found rather more copper in colostrum than in milk. In the milk of three cows the copper in the fat globules varied markedly in individual samples for the same or different cows from 4 to 40 % of the total copper (256). Butter made by the continuous process had a copper content of 0·19 μg./g. (0·12–0·27 μg./g.) compared with 0·27 μg./g. (0·13–0·38 μg./g.) for butter made by the batch process. Tests made at different stages of the process in four plants demonstrated that the initial contamination of cream with copper was largely eliminated during the process (265). The copper content of butter in twenty-four low-ranking factories in the Netherlands was markedly higher than that of butter from forty-four high-ranking factories (263). Methods of reducing this contamination and that of iron (see above) are discussed.

The influence of copper on the development of oxidized flavour in milk and butter is discussed in (257,258,259,261,262,269,270). As with iron, the intensity of the flavour increased with the copper content, samples containing less than 10 μg./100 g. butter being resistant to the flavour (257,259,261,262). The need to avoid copper contamination in butter is emphasized in (257,271). Thompson (272) has demonstrated that with high copper concentrations a copper-protein complex may form in milk which accelerates the 'browning' reaction.

Only a slight flavour was noted in butter when the manganese content was artificially increased above the normal value of 20–25 μg./kg. (257). A method for the determination of zinc in milk is given in (273), and methods for its detection in (266,274). For molybdenum, Archibald (275) quotes a mean value of 7·3 μg. (1·8–14·7)/100 ml. milk, the element being concentrated chiefly in the cream phase. According to him the molybdenum concentration of the milk can easily be increased by giving molybdenum to the cow. Blaxter & Allcroft (276) describe the modifications of their method for the determination of lead in biological materials necessary for milk; a method for the detection of lead is included in (266). According to Gabovich (277) the fluorine content of milk does not appear to be influenced by its level in the drinking water of the cows, but figures reported by Ham & Smith (278) suggest that it may be so related; the authors consider that their evidence is insufficient. Values for the iodine (279,280), bromine (281) and radium (282) content of milk have been published. Paper (280) gives also the iodine content of colostrum, lactational trends in the colostrum and milk of individual cows and seasonal changes in bulk milk from Moscow dairies.

(ii) *Proteins*

Two books (283,284) and a review (285) deal with proteins in general, but contain brief references to milk proteins. Eagles (286) has briefly reviewed their chemistry. The electrophoretic technique has been applied to the study of milk proteins (287,288,289,290,291,292); Heyndrickx & de Vleeshauwer (287,288) have shown differences in protein structure between colostrum, full lactation milk (287) and late lactation milk (288), and Ewerbeck (292) showed differences in the protein

structure of cow's and human milk. Nandi & Rajagopalan (293) have used paper chromatography to confirm the similarity in the amino-acid make-up of cow's milk and of a vegetable milk designed by them. Bigwood (294) quotes provisional values for the essential amino-acids in milk, obtained in his laboratory by chromatographic means; the figures agree satisfactorily with those of published microbiological assays. Modern methods of amino-acid analysis and some analytical values for milk proteins are given in (295).

Values for the essential amino-acid content of cow's milk, determined microbiologically, will be found in (296,297,298) and for the fat-globule membrane of milk in (299). A value for tryptophan (300), determined colorimetrically, is also available. Kugenev (301,302) is of the opinion that the methionine (301) and the arginine, lysine and tyrosine (302) contents of milk are related to the composition of the fodder. A finding more in keeping with the cow's well-known ability to synthesize protein from non-protein nitrogen in the rumen is that of Duncan, Watson, Dunn & Ely (298), who found little or no effect on the amino-acid pattern of colostrum and milk that could be attributed to the feed of the cow; the pattern varied little from the colostral stage to the end of lactation, though the actual concentrations declined. Hansson (303), in a study with monozygous twins, noted, with a high mineral supplement, an increase in the protein produced in early lactation, but in later lactation the production decreased below that of control animals. According to Spisni (304) solvent extraction removes appreciable amounts of methionine from butter.

In experiments with calves Blaxter & Wood (305) obtained biological values of 40–84 and digestibility coefficients of 91–97 for milk proteins; the higher biological values were associated with a higher milk intake. French workers (306) obtained similar biological values for milk proteins with calves, lambs or piglets. A biological value of 90 is reported with rats for the proteins of freeze-dried milk (35). The superiority of milk over rye or the bean *Phaseolus mungo* in restoring growth and increasing haemoglobin in protein-depleted rats has been demonstrated (307). The value of skim-milk protein in the diet of the pig is discussed in (308).

Oser (309) evaluated the nutritive value of proteins by comparing their ratios of amino-acids with those in egg (cf. (310)). In this way he obtained an 'essential amino-acid index' of 90 for milk proteins which agrees well with their biological value. His figures indicate, in agreement with Block & Mitchell's findings (310), that in these proteins the deficit for arginine is similar to that for cystine and methionine together. Oser (309) considers that arginine, rather than the sulphur-acids, is the first limiting factor in milk. This may not be so for the rat or for man in view of their limited capacity to synthesize arginine. The *in vitro* digestibility of cow's milk by trypsin was markedly reduced by the addition of even 5% soya-bean milk; similar effects were obtained with the tryptic inhibitor obtained by extracting soya-bean powder at pH 4 (311). Further data on the rate of digestion of milk, *in vitro*, with pepsin and trypsin will be found in (312).

Interruption of milking for 10 days caused an increase in the total protein concentration in the milk (313). A breed difference in the protein content of milk is reported in (314,315).

(iii) *Fat*

General information on the metabolism of fats, including butter fat, will be found in the publications of a colloquium on lipids held in Paris (316); a new edition of a book dealing with fats and oils (317) contains a chapter on the nutritive value of fats including those of milk.

In the controversy on the relative nutritive value of butter and vegetable fats further evidence for the superiority of butter-fat over arachis oil comes from Jansen's laboratory (318) in a statistical investigation of their published and unpublished findings. The growth-stimulating factor is present in the saponifiable fraction of butter. Given as a supplement to a dry diet butter was found to promote better growth in rats than lard (319). The experiments (320) in which butter was superior to non-vitaminized margarine and inferior to margarine with vitamin A added prove nothing beyond the apparent inadequacy of the basal diet. In a brief report (321) it is suggested that both butter and margarine, which promoted equal growth in rats when included in the diet to the extent of 30%, contain some nutrient apart from true fat present in the milk solids which

accompany the fat in butter, or in the skim milk incorporated in oleomargarine. In experiments with calves butter fat was slightly superior to fresh hydrogenated soya-bean oil (322). On the other side of the balance may be placed the experiments reported in (38), in which no difference was found between the growth of rats on diets containing either butter or cottonseed oil, and those in which growth and fertility of rats was as good with margarine as with butter (323) or as good with a variety of animal and vegetable fats as with ghee (324). A lower incidence of dermatosis in rats was observed with butterfat or lard than with maize oil as supplements to a lactose-containing diet (39). According to (325) the tributirinase activity of rat serum was higher for rats receiving 'crisco' or butterfat than with other (vegetable) fats, all given as 8% of the diet. When the fat content of the diet was 23% the activity with butter was, however, least. Butterfat proved less effective than lard or bacon fat in relieving signs of fat-deficiency in dogs (326, 327, 328, 329). In rat tests (330), and *in vitro* (331), cow and buffalo ghee were better digested than body-fats or various vegetable fats.

A biological method for determining the essential fatty acid content of butter, agreeing satisfactorily with spectrophotometric analysis, is described in (332). Banerjee (333) has confirmed that rats utilize the lactose of milk more efficiently in the presence of fat (cf. (167)) and suggests that on skim-milk diets they excrete galactose because of inability to convert it into glycogen. Rabbits, however, did not excrete galactose under similar conditions.

For cholesterol den Herder & Kruisheer (334) and Nieman & Groot (335) in Holland report values around 11 mg./100 ml. milk, independent of the season according to the former; the latter found that the proportion of the esterified form of cholesterol was higher in summer than in winter.

Much lower values, 2–5 mg./100 ml., are reported from India (336) for Indian breeds of cows and buffaloes. No statistically significant difference due to breed or season was observed. The addition of cholesterol to butter and other fats inhibited fat absorption in rats and guinea-pigs but not in rabbits (337).

B. TREATED MILK

(i) *General*

(*a*) *Heated milk*

In a long-term experiment in McCay's laboratory (337*a*) one group of rats received mineralized pasteurized milk supplemented with cod-liver oil, and three other groups a complete stock diet with either a 10% sugar solution, coffee or water to drink. The rats given milk consumed less calories and attained lower weights than the other rats but were equal to them in skeletal size. Their bones were the best calcified and no decay was found in their teeth, whereas some was present in the other groups. The authors conclude that pasteurized milk supplemented as stated is in all essentials a complete food for the rat.

According to (38), pasteurization (unspecified) had no detrimental effect on the value of milk for rats on wheat-milk diets (see p. 246). Continuous sterilization ('Uperization') by the Alpura process yielded milk equal in total nutritive value for rats to milk heated by the H.T.S.T. method. Both were superior to autoclaved milk (338). Contrary to accepted opinion, Wagner (339) claims that sterilization and even pasteurization bring about deep-seated changes in the nutritive value of milk. His rats on raw milk alone grew in 7 weeks from some 60 to 150 g., on the pasteurized milk to some 110 g., and on sterilized milk they gained only a few grams. Similar differences persisted on bread-milk diets. Schäfer (340) observed poorer growth in piglets given a supplement of boiled skim milk than in litter-mates given pasteurized milk. Extensive information about the content and fluctuations of the major constituents, of calcium, phosphorus, iron, vitamin A, carotene, thiamine, riboflavin and vitamin C in the pasteurized milk supply of six large American cities is contained in (341).

(ii) *Vitamins*

Vitamin A and carotene. It is generally agreed that commercial heat treatment does not affect the vitamin A and carotene content of milk, and fresh evidence for in-bottle sterilization comes from Holland (342, 343). Wagner takes the lonely stand that sterilization destroys most of

the vitamin A and carotene, and maintains his view in uncritical pronouncements (95,339,344) (cf. (345,346)). Becker (94), in one laboratory experiment, found little loss of vitamin A after H.T.S.T. pasteurization, but a 20% loss in another. A second pasteurization followed by boiling produced further loss. On storage for 6 months in the dark or light no appreciable loss of carotene occurred in the Dutch milk (342), but vitamin A decreased by some 20% in the dark and some 40–50% in light.

The vitamin B complex. Wagner (95) reports losses of some 50% of thiamine as a result of sterilization. His claim that riboflavin loses almost as much is more surprising. Heating milk for 5 min. at 75° C. caused no loss of thiamine or riboflavin, but a 10% loss of nicotinic acid (347). Pasteurization caused no loss of thiamine (348), riboflavin (138,348), nicotinic acid (138,348), pantothenic acid or biotin (138). On the other hand, Monzini & Artom (156) observed riboflavin losses of 3·5, 5 and 9% respectively, when milk was boiled for 2 min., pasteurized or sterilized, and Clemow (152) found losses of 4·9–9·5% riboflavin as a result of pasteurization. The possibility that these losses may have been caused by excessive exposure of the milk to light rather than by the heat treatment should not be overlooked.

Values of 66–88 μg. nicotinic acid/100 ml. for pasteurized milk are quoted in (349). No appreciable loss in the pyridoxin content of milk occurred after pasteurization or sterilization, but exposure to light caused some loss (166). There is no evidence that vitamin B_{12} (169,179) or folic acid (169,170) are damaged during pasteurization, the values quoted falling within the normal range for raw milk.

Ascorbic acid. Paper (350) finds the method of Stewart & Sharp (cf. (167)) suitable for measurement of vitamin C in milk in which heat treatment has produced artifacts interfering with the ordinary indophenol titration. Losses in the reduced ascorbic acid content of milk at different stages of the pasteurization process are discussed in (198,199). Loss of reduced ascorbic acid on storage of pasteurized milk was lessened if nitrogen was bubbled through the milk before storage (351), but additions of tocopherol had no appreciable effect (352). Losses of reduced ascorbic acid during storage of pasteurized milk were less severe when copper was added before than when it was added after heat treatment at 121° C. for 15 min. or 104° C. for 20 min.; the reverse occurred in samples heated at 85° C. for 20 min. (353). The stability of ascorbic acid added to milk in relation to iron and copper additions and heat treatment is discussed in (354).

(iii) *Proteins*

A review of the effects of heat treatment on the biological value of milk proteins will be found in (355). A colorimetric method for the detection of amino-acids in milk serum indicated no breakdown of protein during pasteurization (356).

The effect of boiling on major constituents, physical and chemical characteristics, and free amino-acids of milk is considered in (357). Hodson (358), using the rat-growth and rat-repletion techniques, showed that milk proteins in an infant-feeding mixture based on pasteurized milk were not damaged by heating of the preparation immediately before use to either 212° F. for 15 min. or to 230° F. for less than 4–6 min. Pasteurization caused no loss of tryptophan (300). Methods for detecting changes in milk proteins as a result of heat treatment are given in (289,356, 357,359,360).

(*b*) *Irradiated and vitaminized milk* (*all types*)

In the United States, where the method was first commercially developed, the vitamin D enrichment of milk by ultra-violet irradiation has yielded ground to direct addition to milk of vitamin D concentrates (cf. (1)), but in Germany irradiation is still extensively practised. Articles by Wodsak (361) and Thomsen (362) deal with the apparatus and procedure used there, and further details and much useful information about the various aspects of irradiation of milk will be found in Burton's review (363). Several papers or patents describe specifically the Siemens-type irradiation equipment (364,365,366,367). The potency of the irradiated milk by chick test on the milk fat was 14 i.u./g. fat (420 i.u./l. for milk with 3% fat), that is 70% of the highest value

(18·6 i.u./g.) achieved with the same bulk of milk after fourfold irradiation; for the non-irradiated (winter) milk the high value of 2 i.u./g. fat is reported (367). The bacterial count of the milk was incidentally reduced by 70–80%. The Lembke apparatus intended primarily for 'ultra-violet pasteurization' (363,368) may also be used for production of vitamin D milk (363,369). The main wave-length used in this apparatus is 2537Å., and a paper from Lembke's laboratory (370) indicates that its effect on enzymes and other labile constituents of milk is small.

A study (371) of the type of irradiation used by Scheer (cf. (1,362,363)) showed that protein changes become apparent only after irradiation for 20 sec. and are therefore negligible in the 0·5 sec. normally taken in the irradiation of milk by this method.

A patent taken out in connexion with the Siemens process (372) suggests irradiation of either the cream only or of a portion of the milk, the fractions being remixed later. Wagner's statement (227) that irradiation of milk as practised by Scheer in Frankfurt produces no vitamin D whatever in milk is patently untenable, not only because of the evidence to the contrary from Germany (cf. (1,167) and this review), but also because of the vast American experience. The statement has led to a heated polemic (373,374,375,376,377,378,379) which is further considered on p. 276. Wagner (227) is also of the opinion that the chemical method (cf. (1)) used for the assay of the irradiated milk is non-specific. Diemair & Manderscheid (380) have replied to the criticism, and another combined chromatographic-chemical method of estimation has been published (381).

Vitamin D enrichment of milk by addition of suitable concentrates is considered in papers from Russia (382), Sweden (383) and Germany (361,379,384,385,386). In Russia, where apparently vitamin D milk is not yet in use, the addition of irradiated ergosterol in alcoholic solution is considered the method of choice. As vitamin A is easily measured in milk the addition of a definite quantity of it to the vitamin D concentrate is suggested as a check on the final vitamin D potency of the milk (379). Paper (361) gives information about the legal aspects of milk fortification in Germany. Wagner (95) wants to add vitamins A, D and E to milk before it is sterilized to counteract the destruction of these vitamins by the heat treatment he claims to have detected. The addition of vitamins A and D to bottled pasteurized and homogenized milk for special classes of consumer in Switzerland is advocated by Dreyfus (387) who rejects irradiation as a method of enrichment. Vitaminized whole milk constitutes, according to Weckel (388), the greater portion of the liquid milk sales in the United States. Vitaminized skim milk is becoming increasingly popular there, and (388) deals with methods of production, and with flavour and keeping quality; brown bottles or cartons are advised as containers.

Ultra-violet irradiation of milk, apart from the production of vitamin D or destruction of micro-organisms, has been used to destroy riboflavin and thiamine for experimental diets (147,160) or media (389), or to simulate the action of sunlight in the study of seasonal variations in the development of oxidized flavour (390). The use of a germicidal ultra-violet lamp is discussed in (391). γ-radiation in a dosage (146,000 röntgen) sufficient to destroy 99·5% of organisms detectable by plate counts had no effect on vitamin A or riboflavin (392). With higher dosage progressive losses took place in vitamin A, carotene, vitamin E, ascorbic acid and riboflavin, but phosphatase was but little affected (393).

Enrichment of milk for infant feeding by addition of vitamin C tablets immediately before use to obviate loss in handling is suggested in (394).

(*c*) *Soured milk*

The U.S. Department of Agriculture has published a list of references on fermented and cultured milks (395). Most deal with the preparation of such milks, but a few may contain information of nutritional interest. Heupke (396) has described various sour milk preparations, and Davis (397) deals with yogurt in a general article. The possibility that the bactericidal properties of sour milks are due to the presence in them of antibiotics is discussed in (398,399). Kurunga, a Koumiss-like preparation of cow's milk, in which *Bacterium casei* (*Bact. bulgaricum*), *Bact. lactis acidi* (Leichmann) and *Torula curunga* are the main fermentative organisms, is commonly

used by peoples in Soviet western Asia (399, 400, 401). Khundanov (399) tentatively suggests that its curative effect in lung tuberculosis (see also (400, 401)) may be due to the presence in it of nisin. The growth rate of pigs given a supplement of skim milk soured with a nisin-producing organism (*Strep. lactis*) did not differ from that of pigs given a supplement of milk soured with a non-nisin-producing strain of streptococcus (402). Dogs secreted more saliva when given Kefir than with milk brought to the same acidity with lactic acid (403). Rao & Basu (404), in studying the preparation of soured cow's or buffalo's milk (dahi), found that the retention of thiamine, riboflavin and nicotinic acid in the curd varied with the souring organism used. It was best with *Strep. cremoris* with which thiamine increased, riboflavin remained unchanged and there was a slight loss of nicotinic acid. All organisms tested used up some nicotinic acid. The effect of the organism used in the manufacture of yogurt and kefir on the pantothenic acid content of the final product is discussed in (164).

The retention of ascorbic acid added to soured milk is discussed in (405, 406). Yogurt is apparently not suitable for such enrichment, and the rapid loss observed (50% in 24 hr.) is attributed to the action of the lactic acid bacteria (406). Khambatta & Dastur (407), on the other hand, noted only slight loss of the naturally present reduced and total ascorbic acid during preparation of cow or buffalo dahi from raw or boiled milk. They observed no change in the total calcium and phosphorus content, but a marked increase in the dialysable form of these minerals.

In rat tests, in which the mineralized product was given as an exclusive diet, Khambatta & Dastur (408) observed significantly poorer growth with cow and buffalo dahi made from either raw or boiled milk than with freshly boiled milk, and attributed the difference to the formation of ammonia, volatile acids and alcohol in the fermentation. It is of interest in this connexion that Balakrishnan & Rajagopalan (409) obtained better growth with curd (dahi) than with milk when both were added, to supply equal amounts of thiamine, to a basal diet containing all the other B complex vitamins. In further work (410) they showed that the addition of dahi to a poor south-Indian diet produced not only better growth in rats but also higher retention and excretion of thiamine than a supplement of pure thiamine equivalent in quantity to that present in the dahi. From these experiments, and others in which they gave sulphaguanidine to rats (411), they conclude that dahi favours the intestinal synthesis of thiamine. The formation of amino-acids from protein in the process of souring is considered in (356).

(*d*) *Homogenized and soft-curd milk*

Trout (412) has published a book on homogenized milk which includes a section on its nutritive value. The methods, including homogenization, for the production of soft curd milk mixtures for infants are discussed in (413). *In vitro* tests indicated better peptic and fat digestion with homogenized than with ordinary milk (414). Results of a comprehensive inquiry into factors concerned in the development of sunlight flavour in homogenized milk are given in (415, 416, 417, 418, 419). Additions of sucrose and ascorbic acid (420) and the use of brown bottles (421) are suggested for protection against the development of oxidized flavours in homogenized milk. Changes in the pattern of milk proteins after homogenization have been studied by electrophoresis (290, 291).

(*e*) *Milk treated by other methods*

The use of calcium salts and starch in the preparation for infant feeding of boiled milk with a fine protein flocculate is discussed in (422). A patent (423) covers the preparation of non-clotting milk by addition of calcium salts and citrate.

Preservation of milk with hydrogen peroxide caused no change in the total protein content. From extrapolation of results of tests with added vitamin A the authors believe that the loss of vitamin A originally present does not exceed 20% (424).

C. MILK PRODUCTS

(a) *Cheese, whey and buttermilk*

(i) *Cheese*

Poorer growth and reproduction was observed in rats given cream cheese as sole diet than in animals on a stock diet of bread, potatoes, green vegetables, margarine and vitamin A. The addition of green vegetables improved their performance in both respects, but it was still inferior to that on the stock diet (425).

Vitamins. For vitamin A in Polish cheese the following mean values are reported in i.u./100 g.; full fat ser biały 940 (426); Tilsit cheese 1050, Edam 960, Trappist 740 (427); processed cheese 190 (428). In the last paper and, presumably, in the two preceding ones from the same laboratory, the values represent the total vitamin A activity calculated on the assumption that 1 μg. vitamin A $\equiv$ 2·9 i.u. and 1 μg. carotene $\equiv$ 1·7 i.u.

The preparation of skim milk cheese containing edible vegetable or animal fat and supplemented with vitamins A and D is covered by a U.S. patent (429).

The vitamin A and carotene content of Oregon blue vein cheese, produced at monthly intervals throughout the year and ripened for 5 months, varied with the season in the same way as in milk. The mean value (430), calculated as for the nation-wide survey of milk (see (167)), was 1700 i.u./100 g. By comparison with values for Oregon butter, the authors believe that vitamin A was lost neither in manufacture nor in ripening.

Values, in μg./100 g., are available for several vitamins of the B-complex. For thiamine (348, 426, 427, 431) the values vary from some 26 for American cottage cheese (431) to 70–120 for hard cheese (348, 427), and to 90–120 for ser biały and twaróg (426, 427). For riboflavin (151, 156, 348, 426, 427, 431) the range for hard cheese is from 420 to 660 (151, 427), but the value is only 160 in Russian Dutch cheese (348). It varies for twaróg from some 330 to 430 and for ser biały from 450 to 590 (426); the value is some 350 for regular style cottage cheese, but only some 200 for the country style variety, both from California (431). For nicotinic acid (348, 349) the value was 38 in Russian Dutch cheese (348) and 70 for Puerto Rican cottage type (349). Of pyridoxin, Emmental had 560 (166) and there were some 10–16 mg./100 g. of choline in Polish low-fat cheese (432). A loss of thiamine and riboflavin in laboratory-made ser biały when the milk was exposed to sunlight during souring is reported in (426). Paper (348) gives values for changes in thiamine, riboflavin and nicotinic acid in the manufacture of Russian Dutch cheese. During ripening thiamine increased more than twofold, riboflavin was unchanged and the loss of nicotinic acid amounted to 80%. A report of preliminary work on the changes during ripening of Emmental cheese in the quantities of eight vitamins of the B-complex is given in (433). The cheese contained less thiamine and nicotinic acid after 6 than after 3 months' storage, but riboflavin, pantothenic acid, biotin, *p*-aminobenzoic acid, pyridoxin and vitamin B_{12} did not change appreciably. Australian processed cheese stored at 21° C. lost about 10% thiamine after 12 weeks and some 30% after 24 weeks (434) (cf. (167)). Riboflavin synthesis by moulds was responsible for the high riboflavin content of Gorgonzola, Robiola and Crescenza types of cheese compared with hard cheese (156).

American cottage cheese contained no reduced ascorbic acid (196).

Minerals. A method which eliminates ashing, originally designed for the rapid determination of calcium in milk, has been applied to cheese (435), and analysis of seventeen samples covering eight varieties gave values in satisfactory agreement with those of the usual ashing technique.

From Randoin's laboratory in France come reports of extensive investigations into the calcium and phosphorus contents of a large variety of cheese made in France (436, 437, 438, 439). A summary of these findings is given in (440) with analyses done at various stages of the cheese-making process. Of twenty-three varieties of cheese the highest in calcium content on the dry basis was Parmesan (2%), followed by Emmental and Gruyère; the lowest, Brie, Camembert and demi-sel (0·3–0·4%). Mattick (441), in experiments done before the war but only now published, studied the calcium and phosphorus losses that occurred at different stages during the making of Cheddar and Stilton cheese from one bulk of milk. In Cheddar cheese little loss of these minerals

occurred after cutting the curd, and the ripe cheese contained 1% Ca in the dry matter, but in Stilton losses continued until the final stage of manufacture, the ripe Stilton having only 0·1% Ca in the dry matter. The distribution of calcium between the crumb and coat of Stilton cheese was also studied. Polish hard cheeses (426, 427) (Tilsit, Edam and Trappist) contained, in the dry matter, about four times as much calcium (about 2%) and twice as much phosphorus (about 1%) as full cream and half-skimmed ser biały and full cream and three-quarter skimmed twaróg. Orlova (442), in studying preparations of the quarg type, used in Russia medicinally in infant feeding, found 0·4% Ca in rennet quarg and in quarg made from milk coagulated near boiling-point with calcium chloride, but only 0·1% in sour-milk quarg.

Rats receiving Egyptian skim milk cheese, supplemented with cod-liver oil and yeast extract as the sole diet, grew less well than those similarly given one-quarter skimmed (?) cheese and, rather surprisingly, excreted much more phosphorus from a smaller intake and were in negative phosphorus balance (443).

Munch-Petersen (267) found marked variations in the copper content of cheese, 46–61 μg./100 g. for Roquefort and freshly made whole milk cheese and 452 and 675 μg./100 g. for two samples of Emmental cheese.

Proteins. Figures for the total protein content of different types of cheese are given in (426, 427, 431, 444).

Paper chromatography is proving increasingly useful for studying the effect of a number of factors on the content of free amino-acids in cheese (445, 446, 447, 448). Belgian workers (445) quote methionine as only rarely present, but in Sweden (446, 447) it was always detected. These, and other discrepancies, are largely attributed to differences in experimental technique (446, 447).

In tests with different cheeses in the same laboratory definite differences in the amino-acid pattern are noted (448, 449, 450), and small differences in cheeses of the same variety (450, 451).

The effect of ripening on the free amino-acids present in cheese is discussed in (445, 452, 453). Although in any one cheese the same acids were present throughout the process, the relative concentrations changed. No definite correlation was observed between the amino-acids present and flavour in cheese (447, 448). The application of electrophoresis to the study of protein changes during ripening is described in (454, 455, 456). Thomasow (356) describes a colour reaction for the detection of amino-acids in cheese, and a method for tryptophan is given in (300); soft cheese had a lower content of free tryptophan than hard cheese, but the total tryptophan was the same for both varieties.

Block (449) found that the amino-acid pattern of cheese proteins was very similar to that reported for casein, and that this pattern was not affected by pasteurization of the original milk.

As would be expected, the proteins of both skim- and quarter-skimmed (?)-milk cheese were equally digestible for rats (443).

(ii) *Whey (liquid and dried)*

Whey is a valuable animal feedingstuff, but its use is restricted by its high mineral and lactose contents. Means of increasing its value particularly as a source of protein have been studied in several laboratories. Wegelin (457) has used electrodialysis to prepare whey relatively low in minerals and lactose. From Canada come accounts of laboratory (458, 459) and commercial pilot scale (460) methods of increasing the nutritive value of whey by yeast fermentation. *Torula cremoris*, with its ability to metabolize lactose, proved more satisfactory than other yeasts for the production of whey low in lactose and with increased riboflavin and protein (458).

Methods for utilization of whey are given in (461, 462, 463, 464, 465, 466, 467), and its specific use in the baking industry in (468, 469, 470, 471). The use of whey in the feeding of animals, particularly pigs, is discussed in (472), and for pigs only in (48). The increase in the incidence of enlarged hock in young turkeys when 5% dried whey was added to a diet containing 2% fish-liver oil (473) is attributed to the lactose content of the whey; the incidence decreased if the content was reduced (474). The danger of contamination of whey by zinc when stored in zinc containers is noted in (475). Its use as an anti-oxidant for milk is described in (476).

The riboflavin content of whey increased from 175 to 243 μg./100 ml. as a result of 48 hr. fermentation with *T. cremoris* (459). Conditions for the use of whey in the microbiological production of riboflavin with the Clostridium species, *Eremothecium ashbyii*, are discussed in (477), and for production of vitamin B_{12} by *Propionibacterium freudenreichi* and *Lactobacillus bulgaricus* in (478). According to (137) acid whey contained on the dry basis in μg./100 g.: thiamine 380, riboflavin 800, nicotinic acid 1200, pantothenic acid 3100, pyridoxin 420, biotin 20; (479) gives for pantothenic acid a value of 4240. The value quoted in (166) for pyridoxin in liquid whey is high (cf. p. 252). Whey contains an unidentified growth factor for the chick (480, 481, 482) and one (possibly the same) of value to turkeys (474). Briggs & Beeson (483) consider that the 'whey factor' is not essential for the pig since the growth of weanling pigs on an all-vegetable diet was not improved by the addition of 2% whey. Other workers (484) found, however, a growth response when young pigs similarly fed received 4% dried whey and whey fermentation solubles. Vitamin B_T (carnitine) (see p. 253) was demonstrated in whey (189), concentrated in it (485) and finally isolated (188).

The chemistry of the proteins of whey is briefly discussed in (286) and the effect of heat on them in (486). Papers (487, 488) describe the application of electrophoresis to the study of whey proteins. Their amino-acid composition by microbiological assay will be found in (297). The use of spectral analysis for determining the tyrosine and tryptophan content of whey is described in (489), and the discrepancy between values obtained by this method and by chemical assay is discussed in (490). Values for the cystine and methionine contents of whey are given in (491).

Papers (472, 492) deal with the value of whey as a source of high-quality protein for pigs. Its value for the rat as a source of protein was markedly improved when the lactose content was reduced by electrodialysis (457).

Values for the calcium and phosphorus content of whey obtained at different stages in the manufacture of Cheddar and Stilton cheese are included in (441). Values for the iron and copper content of whey cream are given in (251).

(iii) *Buttermilk (liquid and dried)*

Sweet-cream buttermilk powder proved a satisfactory substitute for skim milk powder in bread-making (493), its use in the manufacture of ice-cream is briefly mentioned in (462).

Hoeflake (138) quotes values for the riboflavin, nicotinic acid, pantothenic acid and biotin content of two samples of liquid buttermilk. The value for riboflavin is lower than that for the corresponding sample of raw milk (cf. (494)). A value of 2·7 mg./100 g. for pantothenic acid in dried buttermilk is given in (479). Buttermilk proved a good source of the unidentified factor involved in xanthine-oxidase activity (193). A value of 1·4 mg./100 ml. for total ascorbic acid in fresh buttermilk is quoted in (195).

An electrophoretic study of the proteins of buttermilk is briefly reported in (488), and the detection of amino-acids in it is described in (356) (cf. p. 259).

Values for the concentration of copper in buttermilk are given in (251, 258, 267) and of iron in (251, 258).

(*b*) *Dried and evaporated milk*

(i) *General*

General information on the manufacture, nutritive value and use of dried and evaporated milk is given in (495, 496, 497, 498, 499). Paper (500) deals with methods of utilization of dried milk in Israel. Lange (501) reviews the value of natural or added anti-oxidants for the preservation of dried milk and dried cream; methods for improving the keeping quality and preserving the nutritive value of spray-dried milk are described in (502, 503, 504, 505, 506, 507). Daal-Berg (508) considers that reconstituted roller-dried milk is nutritionally inferior to reconstituted spray-dried milk because of lower calorie, protein and ash content. His analyses did not include the insoluble fraction of the milks.

The use of dried skim milk (493, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518) and of condensed skim

milk(514) as a means of improving the baking qualities and nutritive value of bread continues to attract attention.

Methods for producing the most satisfactory powder for this purpose by regulation of the pre-heating temperature are described in (471,510,519,520). Papers (515,520,521) discuss modifications of the powder and additions to it, and papers (520,522) deal with the effect of dried skim-milk fractions on the baking quality. Analysis of 170 samples of bread in the United States showed that white and enriched breads contained 3·8 parts dry skim milk per 100 parts flour, and whole-wheat and mixed breads 2·4 parts. Milk contributed one-third of the total calcium in the former and one-quarter in the latter(523). Jacquot(513) emphasizes the nutritional benefits of the addition of milk solids, but considers that it might lower the palatability of French bread. He would prefer to have, in France, bread as it is and increase the separate consumption of fresh milk.

The use of dried skim milk in other food products and for culinary purposes is discussed in (517,518,524,525) and its use for the preparation of cheese curd in (526).

Growth tests on rats given a diet consisting of two-thirds wheat and one-third milk (computed on the dry basis) showed that the nutritive value of the milk was not impaired by drying or evaporation(38). Loss of nutritive value for rats of whole dried milk caused by drastic heat treatment (115° C. for 2 hr.) was restored by supplements of vitamin B_{12} or of liver powder(527). Dried whole milk supplemented with dextrin and vitamins was compared in rat-growth tests with a diet intended as a milk substitute for allergic infants and containing the same amounts of protein, fat, carbohydrate, calcium, phosphorus and vitamins but with cooked lean beef as the source of protein(528,529). Trace elements were added to the meat diet, but the milk does not appear to have been mineralized, which may account for the rather poorer growth of the rats on the milk diet. The value of skim milk powder (reconstituted) as a supplement to a poor south Indian rice diet has again been demonstrated in experiments with rats(530). Dental caries in the rat(531) was more pronounced with roller-dried than with spray-dried milk when these constituted 35% of the diet. Autoclaving of the powders increased the cariogenic effect with both(531). In the cotton-rat(532) caries was more pronounced when whole milk was given, as 33% of the ration, in the dry form than when it was reconstituted; some reduction in caries was noted when the milk content of the diet was increased from 32 to 48%(532). In reproduction and lactation tests with rats the nutritive value of an infant breakfast food was markedly improved when its milk content was doubled by replacing 10% of the breakfast food by dried skim milk(533).

In similar tests the value of a bread containing dried milk and other additions was studied(534), and improvement in the nutritive value of white, but not of wholemeal, bread was obtained by the addition of 6% dried skim milk(535). The development of liver cirrhosis in rats given Butter Yellow in a diet of rice and carrots was markedly inhibited by the addition of 15% dried whole milk to the diet(536). Dried whole milk was a rather poor source of a factor necessary for the tolerance by rats of large doses of ergocalciferol(537). Further work confirms the absence in milk powder of the guinea-pig anti-stiffness factor(538,539). Reconstituted whole milk powder, supplemented with iron, copper and vitamins, was used as the basal diet in studies on the relationship between megaloblastic anaemia and scurvy in monkeys(540,541,542). Skim milk powder fortified with vitamins and minerals was used as a basis for a sow's milk substitute for rearing baby pigs(543). Puppies given evaporated cow's milk did not grow as well as those given their mother's milk and had a lower concentration of cholinesterase in the pancreas(544). According to (545) dried skim milk can be left out entirely from English baby chick mashes provided the riboflavin it supplied is given in another form.

(ii) *Vitamins*

The vitamin B complex. Since the last review(1) only a few values for the vitamin content of whole dried milk have come to our notice: riboflavin (Malaya, 1·9 mg./100 g.(151); Italy, 0·7 mg./100 g.(546)), nicotinic acid (England, 900 μg./100 g.(162)), pyridoxin (England, 700 μg./100 g.(165)), folic acid (England, trace(170); U.S. 2·2 μg./100 g.(541); the much higher value of 50 μg./100 g. by rat

assay (547) is of doubtful significance); and vitamin B_{12} (U.S., 3·8 μg./100 g. (179) and 2·5 μg. (541)). In the laboratory preparation of sweetened condensed milk from the pasteurized milk mentioned on p. 259 the loss of thiamine amounted to 14%, of riboflavin to 22% and nicotinic acid lost a further 5% (347). As the condensing was done in a glass vessel the relatively high loss of riboflavin may have been due to the action of light. The same authors report that laboratory drying of whole milk after pre-heating to 85–87° C. and with the air temperature at 165–170° C. caused a loss of 9·6% thiamine and 14·7% riboflavin; nicotinic acid was not affected (347). Values for dried skim milk are: riboflavin (England, 1·8 mg./100 g. (55); Malaya, 3·4 mg./100 g. (151)); pantothenic acid (England, 2·7 mg./100 g. (55); Israel 3·0 mg./100 g. (479)), and vitamin B_{12} (U.S., 3·6 μg./100 g. (174)). Dried skim milk contains unidentified factors for ducks (548) and turkeys (549), and dried whole milk moderate amounts of the factor related to xanthine oxidase (193).

Ascorbic acid. Extensive studies of the ascorbic acid content of evaporated milk have led to the recommendation that for practical purposes it is only necessary in such milk to determine the reduced form (550). The mean value for reduced ascorbic acid in French whole dried milk was 8·4 mg./100 g., in evaporated milk 1·3 mg./100 g. and in sweetened condensed 3·7 mg./100 g. (551). Milk with an initial content of 1·64 mg./100 ml. had 1·17 mg. after pasteurization, and, on the reconstituted basis, 1·06 mg. after condensing and 0·86 mg./100 ml. after final spray-drying (552). It is not stated whether total or only reduced ascorbic acid was measured. Subsequent losses of the condensed milk in frozen storage and of the dried milk at different temperatures and in air pack and nitrogen pack are given in the paper. They were less than in the heat treatment.

Paper (553) reports an extensive study of effects of conditions of pre-heating and storage on the keeping quality of whole dried milks. Preheating to 90–96° C. for 60–80 sec. and storage in nitrogen-pack preserved ascorbic acid best. Added ascorbic acid has been studied as an antioxidant in dried milk (506) or as protein stabilizer (554).

Tocopherol. Values of 34–40 μg./g. fat are reported for whole dried milk and of 23–31 μg./g. fat for evaporated milk (555).

(iii) *Minerals*

Relationships between the keeping quality and copper content of dried milk and the formation of copper-protein complexes are discussed in (272). Dried whole milk in Texas contained 0·29 mg. fluorine/100 g. compared with 0·03 mg./100 g. for New England milk (556). By a spectrographic technique Gehrke & Van Runyon (557) found that the tin content of evaporated milk increased on storage, presumably by absorption from the container, but observed no changes in iron, copper and lead content. A value for the radium content of dried milk is given in (282).

(iv) *Proteins*

Carpenter (558) has discussed in general terms the value of dried milk as a source of protein for pigs and poultry. Values are available for the essential amino-acids in evaporated (449) and dried skim milk (559) and for tryptophan (300, 560) and tyrosine (560) in dried milk. Hostettler & Imhof (561) have studied by electron microscopy structural changes in milk proteins due to processing, and related largely to the degree of heat treatment in evaporated, condensed, roller- and spray-dried milk.

Blaxter & Wood (562), in experiments with preruminant calves, found a biological value of 92 for skim-milk proteins; with pigs a protein-efficiency ratio of 2·5 was found for skim-milk powder (563). Both values are similar to those usually obtained with rats. A new relatively rapid biological method for the evaluation of proteins relating nitrogen intake to liver nitrogen in rats proved satisfactory for dried milk, good relative agreement with the balance-sheet and rat-growth methods being observed (564). De Castro & Pechnik (565) found, rather surprisingly, that mixtures of dried whole milk and maize in which milk supplied 30–50% of the protein had, at an 8% level of protein intake, higher protein-efficiency ratios (2·3–2·6) than milk alone (2·0).

Jones (566), from an examination of his laboratory rat records, confirmed that females withstand dietary deficiencies better than males but, irrespective of the limitations of the diet, found with both sexes an improvement in the value of the proteins of flour by an addition of milk.

The biological value of the proteins of spray-dried whole milk was the same whether the pre-heating was to 190° F. or to 230° F. (567). It remained unchanged in both milks after 6 months' storage in air pack at 37° C. As the keeping qualities of the milk were the same no advantage was gained from pre-heating to the higher temperature. American workers (568) found lower protein-efficiency ratios for pre-heated (2·45–2·50) than for non-pre-heated (2·88) samples of dried skim milk; but no significant difference between milk pre-heated at 63° C. for 30 min. and at 88° C. for 15 min. Drastic experimental pre-heating lowered the value to 2·18. With evaporated milk they found, by comparison with an unsterilized condensed sample, a 5–10% decrease in the value during sterilization. Hodson (569), however, using a rat-repletion method, observed no difference in the nutritive value of proteins between sterilized and unsterilized evaporated milk. Kraft & Morgan (570) found that, with rats, autoclaving caused a marked loss in the protein-efficiency ratio of dried skim milk, mainly by destruction of lysine; with young dogs, however, they observed no such loss. The results of Schroeder, Iacobellis, Lees & Smith (571) at first sight conflict with those just reported, since they observed marked loss in the nutritive value for dogs when skim-milk powder was dry-autoclaved. In their experience, however, reconstituted milk and raw milk (572) could be autoclaved without loss of value for dogs.

The nutritive value for rats of the proteins of evaporated milk was unchanged by storage at room temperature for 12 (568) or 16 (495) months or at 4° C. for 2 years (573). Measurable losses occurred, however, after 1 year at 38° C. or 2 years at room temperature (573) and after 5 years at room temperature (569). Hock (574), in assessing the nutritive value of fodder-yeast proteins, compared them with those of dried milk in rat-growth tests.

Guthneck, Bennett & Schweigert (575), in experiments with protein-depleted adult rats (576), concluded that they utilize 96% of the lysine in milk.

Lang (577) has reviewed the deterioration of proteins in foodstuffs (including milk and casein) by heat or during storage. In further studies with young rats of the deterioration on storage of skim-milk powders (cf. (1,167)) Henry & Kon (578) obtained a marked difference in biological value between stored and fresh samples, but little difference with the same rats when adult because of the smaller lysine requirement of the adult rat. They also confirmed that only lysine suffers loss in gas-pack, but that in air-pack histidine also is affected. Hodson (573), in his study of the effect of storage on the proteins of evaporated milk, noted a loss of about 10% of lysine, histidine and arginine after storage at 38° C. for 1 year, and a loss of about 29% at the end of 2 years; there was then also a 12% loss of tryptophan. In a further paper, Henry & Kon (579) showed that methionine, but not cystine, can make good the original deficiency of both sulphur amino-acids in milk. Addition of large quantities of riboflavin, but not of thiamine or nicotinic acid, improved the retention, by protein-depleted rats, of protein from died skim milk or from casein (580). Utilization of protein from dried milk was not affected by the level of calcium in the diet (581).

Factors affecting browning of evaporated milk, including the Maillard reaction, are discussed in (582), and the application of chromatography to the study of this phenomenon in (583). The formation of copper-protein complexes in milk, with resultant browning, is described in (272), and methods of stabilizing the proteins of dried milk in (554).

(*c*) *Ice cream*

The use of milk in the manufacture of ice cream in the United States is discussed in (462). Paper (196) contains values for ascorbic acid in several varieties of ice cream.

(*d*) *Chocolate milk*

The nutritive value of chocolate milk is considered in a general way in (584).

(e) *Casein, lactalbumin and lactoglobulin*

(i) *General*

Papers dealing only with the non-specific use of casein and lactalbumin as a dietary protein have again been omitted.

A brief review by Marsden (585) on lactalbumin and lactoglobulin lists mainly papers published during the last 5 years.

Mice grew better on a diet containing 85% lactalbumin and 5% casein than on one with 90% casein. Addition of sodium bicarbonate improved the growth of the mice given casein (586). Purified casein proved superior to purified soya-bean proteins for lactation in rats (587), and equal to meat and fish protein for growth and reproduction, but slightly inferior to them for lactation, in mice (588).

The vitamin A activity of diets containing no vitamin A as such or active carotenoids and based on casein and lard, thought to be due to both these components (589,590,591,592), is in all probability due to the presence in lard of a vitamin-A replacing factor (593).

The lipotrophic activity of casein in experimental liver necrosis in rats is considered in (594, 595,596,597), its function in prevention of induced liver tumours in (598,599), and its relation to experimental dental caries in (600). Paper (601) compares casein with other dietary proteins in conferring resistance to infection in rats, and paper (602) compares it with fish protein in the effect on degenerative joint disease in mice. The relation of commercial, of 'vitamin-free' and of hydrolysed casein to cholate synthesis in the dog is discussed in (603). Nicotinic-acid deficiency was produced in cats on a diet containing 35% casein, since they are apparently unable to utilize tryptophan for the synthesis of nicotinic acid (604).

The value of casein as a protein source for farm animals is reviewed in (558), and the value for pigs of lactalbumin obtained as a byproduct in the manufacture of lactose is discussed in (605).

(ii) *Protein*

Perceau (606) has reviewed work on the constitution of lactalbumin and β-lactoglobulin and quotes analytical figures for their amino-acid content. The application of electrophoresis to the fractionation of casein is described in (290,607,608,609,610,611). Chromatographic studies of the amino-acids of casein are reported in (612) and of lactoglobulin in (613). A chromatographic method for isoleucine (614) and a polarographic one for cystine (615) have been used with casein. The application of a manometric method to the determination of L-glutamic and L-aspartic acids in casein is described by Krebs (616), and a microbiological method for L- and D-aspartic acid in casein in (617). Values are reported for tryptophan in casein and β-lactoglobulin (618) and in casein and lactalbumin (619) by chemical methods; methionine in casein and lactalbumin by a chemical method (619); lysine in casein and lactalbumin by a chromatographic method (620); and alanine, glycine and proline in casein and its α-, β-, and γ-components by a radio-isotope technique (621). Less than 0·1% hydroxyproline was found in casein (621), and (by chromatography) no hydroxylysine in casein or lactalbumin (622). The contribution of cystine and methionine to the total sulphur content of casein is considered in (491,623). According to (623) these two acids account for 91% of the organically bound sulphur. Horn, Jones & Blum (559) have summarized values obtained in their laboratory (cf. (1,167)) for ten essential amino-acids in a number of food materials, including casein and lactalbumin. Values are available for eighteen acids in γ-casein (624) and for sixteen acids in β-lactoglobulin (625). Amino-acids have been determined in a dialysed enzymic hydrolysate of casein (626), and in the non-diffusible fraction of a casein hydrolysate (627); the effect of alkali hydrolysis has also been studied (628).

Oser (309) found the agreement between the 'essential amino-acid index' (see p. 257) and the biological value less satisfactory for casein and lactalbumin, for which the index gave higher values, than for milk.

A microbiological measurement of the nutritive value of proteins, based on the growth of *Strep. faecalis* on a pancreatic digest, gave a high value for casein in comparison with the rat-growth technique (629). The authors found that the limiting acids in casein were lysine, arginine,

isoleucine and threonine, in that order, a finding which throws doubt on the value of the method in animal nutrition.

Biological values reported for casein determined by the balance-sheet method at 7–12% levels of protein intake with young rats are: 83 (630), 82 (631), 78 and 84 (632), 73 (633) and 63 (634); with adult rats 61 (633); with pre-ruminant calves 79 (562); with young adult dogs 68 (635). At a 4% level of intake values of 77 and 85 were found with young and adult rats respectively (633). Protein-efficiency ratios of 2·5 (636, 637) for casein and of 3·8 (636) and 1·9–3·9 (637) for lactalbumin are reported.

Casein is suggested as the reference protein in the new method for evaluation of proteins (564) (see p. 266). Unlike it, two other liver-protein methods (638, 639) indicate a superiority of casein over whole-egg proteins; a criticism of these methods will be found in (564). The effects of method of controlling food intake (640), of the strain of mice used (586), of the level of dietary calcium (581), of the dietary fat and calorie intake (641) and of the dietary carbohydrate (642) on the utilization of casein nitrogen have been investigated.

In growth tests with protein-depleted rats, casein was equal to gluten with added lysine and superior to unsupplemented gluten and to zein with lysine and tryptophan (643). Casein enhanced the value of flour proteins (566) and zein (644) for the growth of rats. Information on the improvement of the nutritive value of casein by additions of methionine or cystine or both is given in (597, 632, 636, 645, 646). In self-selection tests rats preferred casein supplemented with methionine to the unsupplemented protein (647). In rat tests, the protein efficiency of lactalbumin was not increased by a supplement of methionine (636); on the other hand, with dogs an improved nitrogen retention was observed with it and also with casein (645).

Lactalbumin again proved (cf. (1)) slightly superior to casein for growth of rats (636, 648), probably because of better balance of the sulphur amino-acids (cf. (310)), a view supported by the findings of Lewis & Fajans (636).

Lysine in casein was better utilized than in lactalbumin (575, 576). Casein proved superior to lactalbumin in measurements by the activity of liver-xanthine oxidase in rats (648), for blood-protein regeneration in anaemic hypoproteinaemic dogs (649), and to groundnut protein for blood-protein regeneration in anaemic rats (650). The better growth of rats on casein than on amino-acid mixtures (651, 652), or on acid (supplemented with tryptophan and cystine) (653) or enzymic (654) hydrolysates of casein is probably to a great extent due to factors other than amino-acid composition. Paper (653) suggests that strepogenin is involved, but (652) denies that it plays a part (see also p. 270).

Pancreatic digests of casein with a higher strepogenin content promoted better growth in rats than papain digests, and strepogenin content is suggested as the most satisfactory criterion for the biological value of protein hydrolysates (655). Casein prepared from 'good quality' dried skim-milk powder had a biological value of 70 compared with one of 65 for casein prepared from a deteriorated powder (656); the protein-efficiency ratio was also reduced. Some loss of nutritive value due to heating has again been demonstrated in animal experiments for casein (637, 646, 657), and for lactose-containing, but not for lactose-free, lactalbumin (637). Losses were accentuated when these proteins were autoclaved with reducing sugars (637, 658, 659). Inactivation of amino-acids other than lysine when casein is refluxed with lactose has been postulated (637), and subsequently confirmed in a study of the rate of enzymic hydrolysis (660). In heated glucose-casein mixtures loss of lysine, arginine and tryptophan was demonstrated by microbiological assay after acid hydrolysis (659, 661) and of arginine, lysine, methionine, histidine and threonine by rat test (659). The greater damage to the protein indicated in the biological experiment may have been due to lesser availability to the rat of amino-acid-sugar complexes that would have been broken down in the hydrolysis that preceded the microbiological assay (cf. (662)). Loss of arginine, and of lysine or histidine or both, was demonstrated by paper chromatography (663). The protein-sugar complexes formed in the autoclaving could be digested with pepsin, chymotrypsin and pancreatin but not with trypsin or papain (664).

Lea and his colleagues have continued their studies on the chemical nature of the 'browning

reaction' in casein (665, 666, 667). Lysine was not appreciably involved in the reaction when casein was heated with glucosamine (665, 667). A 'browning reaction' involving copper-protein complexes is discussed in (272, 668).

(iii) *Impurities in casein of nutritional significance*

The purification of casein for nutritional studies is described in (669). The crude and purified products contained, in μg./100 g., thiamine 145 and 12, riboflavin 280 and 20, pyridoxin 120 and no detectable amounts, nicotinic acid 320 and 35. The comparison in nutritive value between crude and purified casein in (670), said to be in favour of the former, cannot be interpreted because of the marked disparity in the initial weights of the rats.

Values of 1·5–11 μg. biotin/100 g. commercial casein are reported in (671). They varied according to the micro-organism used in the assay and the concentration of acid used for hydrolysis, the vitamin probably being present in a bound form. Crude casein contains 0·1 μg. vitamin B_{12}/g. (672) and 'vitamin-free' casein 0·02 μg./g. (652).

Kodicek & Mistry (673) have described a microbiological method for the assay of strepogenin; 'vitamin-free' caseins contained very little, casein hydrolysates none or traces, but lactoglobulin was a fairly good source. Karrer & Pfaltz (655) found no strepogenin in acid-hydrolysed casein but appreciable quantities in enzyme-hydrolysates. It is now considered (cf. (1)) that casein contains traces of the factor necessary for the maintenance of the xanthine-oxidase activity of rat's liver (674, 675). The presence is reported in casein of unidentified factor(s) for rats (676, 677 (possibly vitamin B_{12}), 678, 679), turkeys (549) and the organism, *Eremothecium ashbyii* (680). The importance of good-quality extracted casein in the study of the vitamin requirements of the rat is stressed in (681).

(*f*) *Lactose*

Inter-relationships between fat and lactose in their utilization by the rat are discussed in (39, 333, 682, 683), adaptation to high lactose diets in (683, 684), and a relationship between milk lactose and the cerebroside content of the brain in (333, 685). The excretion of galactose in the urine by rats receiving skim-milk diets may be due to their inability to convert it to glycogen in the absence of fat. However, the formation of cerebrosides and liver fat is apparently unaffected. The mechanism is probably different in rabbits as they do not excrete galactose when given skim milk (333).

In the experiments (528) mentioned on p. 265, in which a meat diet was found superior to milk, addition of lactose to the meat diet to the amount present in milk depressed the growth of rats below that on the milk diet.

Lactose promoted the synthesis of lysine and histidine in the gut of the rat (642), of riboflavin, biotin and folic acid in the cotton rat (686), and spared vitamin B_6 in chicks receiving it in sub-optimal amounts (687).

Lactose is not involved in the 'browning reaction' occurring with copper-protein complexes (668). The use of lactose in bread-making is discussed in (470).

IV. NUTRITIVE VALUE OF MILK FOR MAN

A. GENERAL

(*a*) *Introduction*

The comprehensive statistics of the Commonwealth Economic Committee (688, 689) indicate that the rise in world production of milk noted in the last review (1) has not been maintained, although the level is still some 8% higher than before the war. In general, the consumption of liquid milk has increased at the expense of processed milk (including cheese). Further detailed information is available in F.A.O. publications (690, 691, 692, 693, 694, 695, 696). The last two are monthly journals of which (696) has now replaced (695) and appears in separate English, French and Spanish editions. Brief summaries of the world position will be found in (697, 698).

Information about production and consumption in Venezuela is given in (699), for French West Africa in (700) and for the United States in (701, 702, 703, 704, 705, 706, 707, 708, 709). Publication (704) quotes annual figures for each State for the first half of this century, and (707, 708, 709) deal with individual States. Hollingsworth (710) has summarized trends in consumption for the United Kingdom, and further information is available in the monthly publication of the Central Statistical Office (711). Further publications deal with Australia (712, 713, 714, 715, 716, 717, 718, 719, 720), New Zealand (721, 722, 723) and Canada (724, 725, 726). Paper (715) contains also information from other Commonwealth and foreign countries.

Publication (727) reviews post-war trends in western Europe, (728) gives consumption figures for the O.E.E.C. (Organization for European Economic Co-operation) countries, and (729) the pre-war and present-day consumption in several northern European countries, North America and Australia. Consumption figures for different towns or districts are given for Holland (730), Germany (731, 732), Switzerland (733, 734) and Italy (735, 736). Low milk intakes are reported for Belgium (737), Greece (738), Israel (500), India (739, 740) and Pakistan (741). In Israel (500) steps are being taken to increase consumption: dried skim milk is imported, reconstituted and mixed with the available fresh milk to give two mixtures, one containing 0·85% fat, freely available in stores and beverage bars, the other with 2·34% fat rationed for home consumption.

Owing to the high price of butter and the relative cheapness of margarine, the consumption of butter in the United States has declined, and that of margarine increased. At the same time the government there has accumulated very large stocks of butter by purchase in support of price (see, for example, (742, 743)).

The contribution of 0·5 l. (0·9 pint) of milk daily to the requirements of children and adults for protein, minerals and better known vitamins is tabulated in (5), and the value of milk as a cheap source of calcium, protein and riboflavin in Venezuela is stressed in (744).

Problems of the supply of dairy products to the U.S.A. armed forces are discussed in (745).

(*b*) *Nutritional surveys*

As in the past, only some of the many publications are quoted. Mork (746) has reviewed the literature on factors affecting milk consumption throughout the world, and a brief review of the position in the United States is given in (747). A second world-food survey by F.A.O. (748) (cf. (167)) includes information about milk consumption. Consumption in Norway is comprehensively reviewed in (749), and paper (750) gives information for the Scandinavian and some northern European countries. In the western zone of Berlin the consumption of milk was well below that of the lowest income group in Germany in 1927–8 and only half that of civil servants at the same time (751).

Milk consumption usually increases with the income; confirmation of this relationship will be found in (729, 749, 752, 753, 755), with statistical support in (749, 752). In the United Kingdom (729) differences between income groups were less in 1950 than before the last war as those with smaller incomes consumed relatively more. In Puerto la Cruz in Venezuela (756) less than 10% of the total expenditure on food was spent on milk, but in the United Kingdom (729), in Groton township, New York (757) and in home management houses at Purdue, Connecticut (758), the proportion was some 16–18%. Of interest is the comparison between the food budget of an American clergyman's family in 1830, who spent 3·7% of their total food expenditure on milk, with that of 97 families with comparable income in Birmingham (Alabama) in 1948 who spent 12·1% on milk in its various forms (759). The relationship between income and milk consumption and the effect of the removal of food subsidies in Sweden is discussed in (760). In Czechoslovakia the consumption of butter increased with the income but much less steeply than that of lard (761). Miners' households in Slovakia consumed much less butter than those in Bohemia and Moravia (762).

Comparisons of actual milk intakes with those recommended for children by the (U.S.A.) National Research Council (about 1½ pints*) are available from Louisiana, where according to

* Unless otherwise stated the Imperial pint (568·2 ml.) is meant.

one report (763) 74% of the children in one community reached the recommended level, though another report from the same place (764) puts the percentage at between 50 and 60. In Toronto (765) one-quarter of the children at two suburban schools took less than one-half of the quantity of milk recommended in Canada's Food Rules (cf. (766)).

Milk and ice cream were among the most popular foods with Nebraskan children (767) and with boys of 17–19 years in California (768), buttermilk was unpopular in both places, and evaporated milk in California. In Alaska, however, where fresh milk is almost unobtainable, evaporated milk was readily taken by the native Indian children (769). A Newfoundland survey compares diets eaten in an orphanage with those eaten by children (and adults) in St Anthony and its outposts (770). The orphanage diet provided a child with milk and cheese equivalent to 35 oz. milk daily, whereas only small amounts were consumed in St Anthony where the nutritional status was much poorer. Addition of milk is recommended as the most satisfactory method of improving the St Anthony diet. The nutritive contribution of the additional milk taken with the school lunch by children in a Maryland town is discussed in (771). Inclusion of milk in children's canteen meals in France is recommended in (772).

Two valuable reports of the Ministry of Food (773, 774) give detailed information about the consumption of milk in England in 1940–50, its nutritive contributions and the working of the National Milk Scheme. The mean daily consumption of milk by all householders in 1950 was two-thirds of a pint per head. A comparison of the M.O.F. data for 1940–9 (773) with those of the Institute of Statistics in Oxford will be found in (775). A survey in Iowa in 1946–7 (776) with young and middle-aged people has yielded interesting information about factors influencing milk consumption. Reports of nutrition surveys carried out in different areas of France include information about such factors (777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788), and paper (789) deals specifically with consumption of dairy products by peasants in six regions of France and contains much of interest. Much more milk was consumed in the east than in the west of France. Paper (790) compares expenditure on, and consumption of, milk, cheese and butter in England and in France. In North Carolina milk consumption in rural districts (791) was about double that in urban districts (792). Papers (793, 794, 795, 796) give the results of a 1-week survey on the use of dairy products by families in Oakland and Los Angeles, and (797) summarizes the findings. Papers (778, 793) confirm that milk intake decreases with age. According to (793, 798) American men consume more milk than women at all ages, but no differences are reported in a British survey of elderly people (799).

A full report (800) has appeared of the study of the preference of Californian boys for milk bread with higher milk content mentioned in the previous review (1). From Holland information is available about milk consumption of the population of Uden (801), and of pregnant women in rural districts, less than 10% of whom took as much as 1 l. of milk daily (802). In a milk-consumption survey in Denver, Colorado, 53% of those questioned considered milk the most important food for maintenance of health (803).

(*c*) *General effects*

In a general article Mohr (804) considers the composition, nutritive value, vitamin fortification and the hygienic quality of milk suitable for infants and children. Treatments or modifications of cow's milk for infant feeding include homogenization and vitaminization (386, 805), flocculation of casein (422), addition of fruit juices (806, 807) and preparation of a 'bifidus milk' (808). Kalliala (809) has studied *in vitro* the peptic digestion of many modifications of cow's milk for infants including the Finnish sour milk piimä, and has reviewed the literature on the subject. Baby foods are described, based on dried milk (810, 811, 812) or dried whey (813, 814). A patent (815) covers the preparation of a human milk substitute. A substitute for buttermilk for infant feeding is described in (816). Klinke (817) has discussed the value of a number of milk preparations for artificial feeding of infants in use in Germany, and Neyroud & Du Pan (818) made a similar study of Swiss products. Preparations with cow's milk suitable for premature babies are considered in (819, 820, 821, 822, 823, 824), and Morf (825) has compared the value of buttermilk with that of

protein milk as additions to breast milk for such babies. The effect of added carbohydrate on the renal water requirements of infants given evaporated milk mixtures is considered in (826). A conference of The Nutrition Society dealt with the feeding of normal and premature infants (827).

Milk therapy has figured largely (828, 829, 830, 831) in the treatment of kwashiorkor, a tropical protein-deficiency disease of children (828, 832, 833). The disease does not appear among African people consuming much milk (828), and the incidence in Fiji was higher in Fijian infants weaned on to largely carbohydrate diets than in Indian infants weaned on to diets containing milk (834).

Mehlnährschaden, a disease now believed to be closely related to kwashiorkor (828), that appeared among infants during the siege of Budapest in the last war, responded dramatically to milk in sufficient quantities, but if the milk was discontinued infants cured of the associated oedema died sooner than those untreated, and small quantities of milk were detrimental (835). Experience in Jamaica (836) is perhaps similar in indicating that small quantities of milk in a diet deficient in protein in no way protected children from liver disease. Paper (837) should also be consulted in this connexion.

The use of skim milk and avoidance of butter in coeliac disease is considered in (838).

A caramelized lactose-butterfat-buttermilk was used with success for infants suffering from acute and chronic dystrophy and dyspepsia (839), and milk in very large quantities for children with nutritive failure (840, 841, 842). Adults obtained relief from gastric acidity by sucking tablets of dried whole milk and antacids (843), and from various intestinal disorders by therapy with β-lactose (844). Paper (845) considers the relation of respiratory infections of infants to bottle-feeding, and paper (846) the methods of handling and preparation of milk for infant feeding in relation to infantile diarrhoea in Finland. Buchan (847) considers it possible that a milk-borne allergen is a fundamental factor in the aetiology of rheumatic fever in children.

Several papers deal with milk allergy in infants, children and adults. Its incidence and means of detection are discussed in (848), and Clein (849), in a clinical study, considers that the condition is more common than is generally realized. It is, however, seldom responsible for respiratory allergy (850). Replacement of cow's milk by a soya-bean milk has proved successful in the relief of this condition (849, 851) and the infants could later be given milk again (851). Detection of gastro-intestinal allergy due to milk is described in (852). Paper (853) ascribes classical migraine to food allergy and lists milk as the second most important allergen. In a case of milk allergy desensitization was achieved in 2 weeks by graded small quantities of milk in water (854). A 4-year-old boy allergic to cow's milk from 7 months could take boiled milk with impunity (855), and boiling of cow's milk or substitution by goat's milk in allergic conditions is advocated in (856). A mineral-enriched meat diet proved a satisfactory substitute for milk for an infant suffering from cow's milk allergy and for one suffering from galactosaemia (529).

Weighing and analysis of the food of four children in an American orphanage showed that milk contributed only 18% of the total calories, but 42% of the protein, 22% of the thiamine, 67% of the riboflavin and 81% of the calcium, and also 12% of the vitamin C (857). In a similarly exact study of American school lunches, half a pint (American) of milk contributed 25% of the calories of the lunch and 30% of the protein, 25% of the thiamine, 50% of the riboflavin and 60% of the calcium (858).

Milk given as a mid-morning snack frequently spoilt the children's appetites for their mid-day meal (859, 860). The suggestion in England (859) is to give it earlier, in Switzerland (860) to replace it by an apple. Milk proved of value in allaying irritation and vomiting after large doses of aureomycin by mouth (861). An inquiry into the incidence of digestive disturbances with a large number of Spanish foods indicated that milk and cheese were well tolerated by over 90% of the 550 people interrogated (862). Andross (863) considers the culinary uses of milk and its nutritive contributions.

B. MILK COMPONENTS

(*a*) *Vitamins*

Milk and milk products contribute just under 20% of the vitamin A of the food in this country (773, 774) and rather more in Australia (718, 719, 720). In California (795) dairy products supplied over 35% of the allowances recommended for vitamin A by the (U.S.A.) National Research Council.

Haubold (864) compares in general terms cow's milk and breast milk as sources of fat-soluble vitamins for the infant. Infants absorbed vitamin A much better from milk than from an oily solution or from liquefied butter, though not quite as well as from an aqueous dispersion. The absorption was inversely related to the particle size of the vitamin A carrier (865). Vitamin A absorption by premature infants was not affected by the level of butter-fat in their diet (866). Papers (92, 867) postulate a relationship between endemic goitre and a low vitamin A intake, and blame the reduced winter intake of vitamin A in Germany due to seasonal and other causes for increased incidence of the disease there.

The excellent availability of riboflavin in ice cream reported in the previous review (1) has been confirmed in further experiments with young women (868).

In England milk and milk products supplied some 10% of the thiamine and some 40% of the riboflavin in the food (773, 774). In California (795) dairy products supplied some 18% of the thiamine allowance, and about 70% of that of riboflavin, recommended by the (U.S.A.) National Research Council. Chalmers (869) gave breast fed and artificially fed infants during the first 10 days of life a supplement of thiamine, riboflavin and nicotinamide as he suspected on theoretical grounds that their intake might be inadequate. Not surprisingly the addition proved unnecessary (cf. (870)). Pett (871), in a brief summary, reports dramatic improvement in riboflavin, vitamin A and nicotinic acid nutrition and in general health of pupils in a residential school in Canada when their milk intake was trebled. The value of milk as a source of protein and riboflavin for tubercular bed patients is considered in (872). Evidence of intestinal synthesis of vitamin B_6 in infants given cow's milk is presented in (873). The possible inhibition of intestinal synthesis of vitamins by the presence in milk of antibiotics is suggested in (874).

With a milk diet the microbiological vitamin B_{12} activity of the urine of young adults was equal to that on a normal mixed diet, but those of folic acid and the citrovorum factor were lower (875). Fresh milk or concentrated whey were inadequate sources of Castle's intrinsic factor for patients suffering from pernicious anaemia (876).

(*b*) *Proteins*

Dairy products supply some 20% of the protein of the food, both in this country (773, 774) and in Australia (718, 719, 720). In California (795) they supplied over 40% of the allowance recommended by the (U.S.A.) National Research Council.

In experiments with Indian volunteers (877) the biological value of the proteins of a largely vegetable diet with some milk was, surprisingly, superior to that of a diet supplying the same amount of nitrogen but containing three times the amount of milk and also eggs.

The contents of essential amino-acids in cow's milk, breast milk and a malted baby food are compared in (878). Albanese (879) discusses the amino-acid composition of various natural and artificial milks for infants in relation to their amino-acid requirements. He considers that the protein make-up of breast milk is inadequate for the proper nutrition of infants beyond 3 months, and that it can be improved by supplements of cow's milk and of meat.

Leverton and her collaborators have continued their studies of the retention of nitrogen by young women taking milk with different meals (880, 881).

The value of milk in kwashiorkor has already been discussed on p. 273. Papers (830, 833) deal more specifically with some protein aspects.

Klingemann (882), in Germany, advises a lactalbumin preparation for the treatment of patients with protein deficiency and of persons returning from prison camps. In India lack of milk

protein in their diet is blamed for nutritional dystrophy observed among 150 Madras children (883). In Mexico undernourished children recovered equally well on a vegetable-protein diet as on a diet containing milk and eggs, though they regenerated plasma protein more slowly (884). Vegetable substitutes for milk, mostly based on the soya-bean, are considered in (885, 886, 887). According to (888) a purely vegetable diet led, in a young volunteer, to a reduction in serum albumin; a return to normal levels followed the addition of milk to the diet. In an experiment in Germany casein proved effective in restoring the working capacity of two miners after it had declined on a low-protein diet (889).

Nasset & Tully (890) have studied the urinary amino-acid excretion of healthy young men and women on diets containing different proteins, including casein, and found no correlation with the biological value of the protein. However, in a study of patients with gastro-intestinal disease (891) in which meat and eggs, dried skim milk, evaporated milk and an enzymic liver digest were compared, excretion was higher with the hydrolysate, and there was more arginine and lysine in the urine with the evaporated than with the dried milk. The metabolism of the sulphur amino-acids of infants receiving different milk mixtures is considered in (892), and Ross (893) deals with the faecal amino-acid excretion of breast-fed and artificially fed infants.

Premature infants utilized equally well milk mixtures supplemented with casein as those with casein hydrolysate (894), and meat protein as efficiently as milk protein (895).

A protein hydrolysate with milk proved superior in buffering capacity for peptic ulcer patients to either component alone (896), and whole or skim milk were better than a protein hydrolysate for the same purpose with two healthy adults (897). The use and value of casein hydrolysates given by mouth (898, 899) or by injection (899, 900, 901) is considered.

(*c*) *Minerals*

Dairy products supplied some 50% of the food calcium in the United Kingdom (773, 774); in Australia, where the total intake was lower, some 75% (718, 719, 720). In California the contribution amounted to some 70% of the N.R.C. recommended allowance (795). In Belgium, where the calcium intake is said to be low, increased consumption of dairy products is urged (737).

Though the absolute amounts of calcium and phosphorus are much higher in cow's milk than in breast milk, the Ca:P ratio of cow's milk is lower. It is often further reduced in milk preparations for babies by the addition of phosphates in the evaporation of milk. Paper (902) attributes neonatal tetany in artificially fed infants to this relative surfeit of phosphorus, and suggests dilution with water and addition of calcium to cow's milk given to young infants. Gittleman & Pincus (903) observed that whole milk mixtures led to hyperphosphataemia and hypocalcaemia in many infants and that evaporated milk was even worse in this respect, but paper (904) reports hyperphosphataemia but no hypocalcaemia with it.

Mellander (905) found that calcium bound to phosphopeptides from casein, and given by mouth, promoted calcification in rachitic children, sometimes even in the absence of vitamin D.

With diets in which nearly 50% of the calcium was derived from milk Steggerda & Mitchell (906) found a lower requirement for calcium equilibrium of the adult male, 7·40 mg./kg. body weight/day, than that, around 9 mg., reported by them earlier (907, 908). Working in Lima with adult males not necessarily adapted to low calcium intakes Hegsted, Moscoso & Collazos Ch. (909) found with diets containing only very little milk even lower needs for calcium, around 2–4 mg./kg./day (cf. (1, 167)). They are of the opinion that when calcium supplies are limited they should be reserved for children and for women in the child-bearing age.

The dangers of too much milk for adults are illustrated by the case (910) of painful 'calcium gout' in a man of 56 who was in the habit of taking a gallon of milk daily and recovered to a great extent on giving it up.

Belgian milk contains about as much iron as breast milk (see (254) p. 256), their milk mixtures for babies often 3–4 times more (257). Information about the contribution of milk to the cobalt intake of college women in Texas is given in (911).

(*d*) *Fat*

Premature infants retained fat less efficiently from cow's milk than from breast milk (912); there was no significant difference in the retention of cow's milk- or of meat-fat (895). The replacement in a baby food, Biolac, of milk fat by a fat nearer in composition to that of breast milk did not alter its nutritive value for infants (913). Steggerda & Mitchell (906) found that in adults the amount of butter fat in the diet had no effect on calcium retention.

C. EFFECT OF TREATMENT

(*a*) *Effect of heating and processing*

Evaporated milk diluted with water and supplemented with vitamins proved a very satisfactory food for premature (914) and normal (915) infants. The preparation of dried milks suitable for the production of reconstituted milk of good flavour and readily acceptable is discussed in (916, 917).

(*b*) *Irradiated and vitaminized milk* (*all types*)

The controversy now going on in Germany about the antirachitic value of irradiated milk has already been mentioned on p. 260. Scheer (373, 376) has answered the criticism of Wagner (227, 374) and of Theopold (375) that such milk is of no value for infants, Graser (918) has published results of clinical tests in Frankfurt showing its good effects there, and the Frankfurt method of general prophylaxis by irradiation of the milk supply is also praised by Hellbrügge & Vogt (378) and thought useful by Hövels, Rasche, Bock & Sommerhage (919). Though recognizing the value of irradiated milk, paper (385) considers direct addition of vitamin D to milk technically easier and preferable, and Berthold & Schultze (920) in Hamburg and Adam & Krafczyk (379) in Erlangen consider it superior on clinical evidence.

Further results of tests in Germany with milk enriched by addition will be found in (386, 414, 921, 922), of tests in America in (915), and in England in (923) where the enriched evaporated milk supplemented with vitamin C proved, in general, very satisfactory, though the incidence of respiratory infections was higher in infants so fed than in others breast-fed or given dried or whole milk, in all three instances with additional vitamin A. A pronouncement by the American Medical Association on the value and use of vitamin D milk in the United States will be found in (924).

Braestrup (824) has reported from Denmark on the use for premature and normal infants of a baby food fortified with vitamins A, C and D, thiamine and iron.

(*c*) *Soured milk*

Heupke (396) discusses the therapeutic applications of various sour milk preparations, and Davis (397) the value of yogurt in human nutrition. Clinical tests of it on infants with nutritional disturbances are reported by Mayer (925), who (926) as well as Adam (927) has also studied 'bifidus milk'. Both authors claim that the milk rapidly establishes *Lactobacillus bifidus* in the intestine and obtained excellent results with it in infants.

According to Khundanov (399, 401) kurunga (see p. 260) given to patients in quantities of 250–1000 ml. daily is markedly effective in tuberculosis of the lung.

A German patent (928) describes the preparation of milk clotted and acidified by the addition of a mixture of citric acid and calcium citrate and claims antirachitic properties for it.

(*d*) *Homogenized and soft-curd milk*

Jochims (929) has reviewed the value of homogenized milk in infant feeding. He believes that homogenization neither improves nor impairs the nutritive value. With healthy infants Säger (930) found better absorption of fat from lypolysed than from homogenized milk and doubts whether homogenization brings cow's milk nearer to breast milk in its physical properties.

S. K. KON
K. M. HENRY

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING,
SHINFIELD, NR. READING

checked by
HG 7/7/54

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